

LAW AND ORDER IN WHEAT FLOUR DOUGH

Colloidal Aspects of the Wheat Flour Dough and its
Lipid and Protein Constituents in Aqueous Media



THOMMY L-G. CARLSON

Lund 1981



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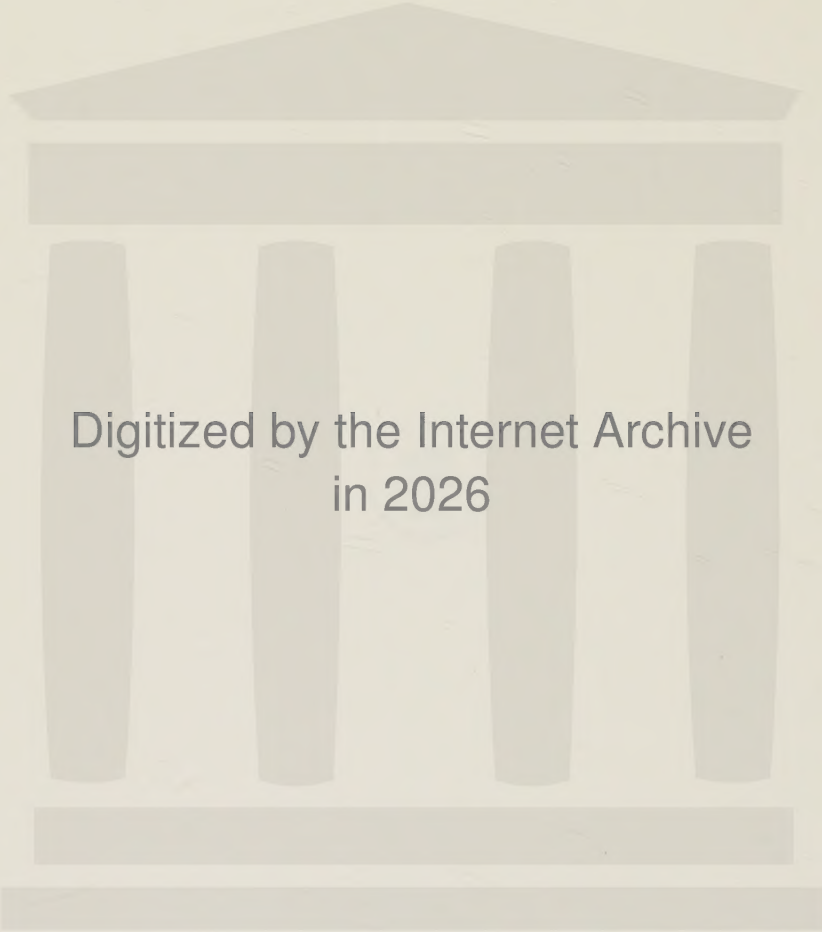
THOMMY L-G. CARLSON



LUND 1981



TO
THE CRAFTSMAN WITH HIS HAND
THE HIDDEN SECRETS OF WHICH
WE STRIVE
TO REVEAL AND UNDERSTAND



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PUBLICATIONS

This thesis is based on the following papers, which will be referred to by Roman numerals in the text.

- I Phase equilibria and structures in the aqueous system of wheat lipids.
T.L-G. Carlson, K. Larsson and Y. Mieziš
Cereal Chem. 1978, 55, 168-179.
- II Phase equilibria in the aqueous system of wheat gluten lipids and in the aqueous salt system of wheat lipids.
T.L-G. Carlson, K. Larsson, Y. Mieziš and S. Poovarodom
Cereal Chem. 1979, 56, 417-419.
- III Physical structure and phase relations of aqueous systems of lipids from rye and triticale in relation to wheat lipids.
T.L-G. Carlson, K. Larsson and Y. Mieziš
J. Disp. Sci. Techn. 1980, 1, 197-208.
- IV Monolayer characteristics of A-gliadin, a wheat storage protein, at the air/water interface.
T.L-G. Carlson
- V Free surface energy in the elasticity of wheat flour dough.
T. L-G. Carlson and L. Bohlin.
Cereal Chem. 1978, 55, 539-544.
- VI Cone plate instrument for stress relaxation measurements.
L. Bohlin, T.L-G. Carlson and G. Bäckström
J. Colloid Interface Sci. 1980, 73, 61-65.
- VII Shear stress relaxation of wheat flour dough and gluten.
L. Bohlin and T.L-G. Carlson
Colloids and Surfaces 1980, 2, 59-69.
- VIII Dynamic viscoelastic properties of wheat flour dough - Dependence on mixing time.
L. Bohlin and T.L-G. Carlson
Cereal Chem. 1980, 57, 174-177.

CONTENTS		page
1	INTRODUCTION	9
1.1	General	9
1.2	The present study	10
2	MICRO-STRUCTURE AND CHEMICAL COMPOSITION OF WHEAT KERNALS	10
2.1	Micro-structure of wheat	10
2.2	Chemical composition of wheat	12
3	WHEAT LIPIDS AND PROTEINS IN AQUEOUS BULK PHASE AND AT THE AIR/WATER INTERFACE	19
3.1	Surface behaviour	19
3.1.1	Wheat lipids	19
3.1.2	Wheat storage proteins	20
3.1.3	Wheat lipid and protein	21
3.2	Aqueous bulk behaviour	24
3.2.1	Wheat lipids	24
3.2.2	Lipids from different wheat cultivars and from rye and triticale	32
3.2.3	Wheat storage proteins	32
3.3	Discussion regarding the conformation or structure of wheat storage proteins	41
4	COLLOIDAL NATURE OF WHEAT DOUGH AND GLUTEN	41
4.1	Dough and gluten as colloidal dispersions	41
4.1.1	Wheat storage proteins	42
4.1.2	Wheat lipids	43
4.1.3	Equilibrium spreading pressure of wheat constituents	44
4.2	Rheology of dough and gluten	45
4.2.1	Flow behaviour	45
4.2.2	Flow behaviour and structure	51
4.3	A qualitative colloidal model of wheat flour dough	60
5	COLLOIDAL NATURE AND BAKING PERFORMANCE OF WHEAT FLOUR DOUGH	60
5.1	Introduction	60
5.2	Dough and loaf volume	62
5.2.1	Effects of foam stabilizer and destabilizer	62
5.3	Loaf volumes and flow properties	71

6	SUMMARY	75
7	SAMMANFATTNING	77
8	ACKNOWLEDGEMENTS	78
9	IMPORTANT SYMBOLS AND ABBREVIATIONS	79
10	REFERENCES	80

1 INTRODUCTION

1.1 General

Wheat flour dough is a very complex material and in order to understand the dough behaviour, as it is used in baking and other technical applications, both a chemical and a physico-chemical approach is needed. The chemical approach has dominated cereal research during the last three decades, and has resulted in important information on the composition of cereals. The physico-chemical knowledge of the cereals, however, is still very limited and therefore also the knowledge of their functional properties.

The physical properties of a material in general depends on its actual physical state of aggregation - vapour, liquid and solid - all relevant to the dough system. Material at a boundary between two homogeneous phases shows physical properties different from those of the bulk materials. Just as in a bulk phase, the molecules at an interface may exist in solid, liquid and gaseous states, i.e. there exists different types of interfacial states. A correspondence between bulk and surface states has been shown to exist for lipids (Lundqvist, 1978). Systems, where the surface area is large compared to the bulk volume, belong to the colloidal systems. These systems can be classified in three groups (Shaw, 1970):

1. Colloidal dispersions, which are thermodynamically unstable due to their large free surface energy. These systems consist of two or more bulk phases which are separated by interfaces. Examples of simple colloidal dispersions are foams and emulsions. In these the continuous phases are liquids, and the dispersed phase is a gas and a liquid, respectively.
2. Association colloids, which are thermodynamically stable systems. Examples of association colloids are micellar solutions.
3. True solutions of macromolecular material, which are thermodynamically stable systems.

Factors that contribute to the overall physico-chemical nature of a colloidal dispersion are the physical properties of the continuous phase, the dispersed phases and the interfacial structures.

Very few studies deal with the colloidal properties of cereal doughs and of dough components. Bungenberg de Jong and Klaar (1929) were among the first to report on the colloid chemistry of wheat flour proteins. In a number of papers (Bungenberg de Jong, 1932; Bungenberg de Jong and Klaar, 1930a,

1930b, 1931, 1932, 1952) they described the colloidal behaviour of protein drops obtained by dispersion of wheat protein in acidic solutions. The air cells in dough and their significance for the baking process have been studied by Baker and Mize (1941), Baker (1941), and Sandstedt et al. (1954). Hess (1954) was the first to report studies of low angle X-ray diffraction and electron microscopic techniques on cereals. He discovered a diffraction spacing in wheat gluten at ≈ 55 Å. Traub et al. (1957) and Grosskreutz (1960) attributed this low-angle diffraction spacing to the presence of bimolecular leaflets of lipids. Grosskreutz (1961) also proposed a model of the structure of gluten. MacRitchie (1976) has reported on the foaming behaviour of the aqueous phase of wheat flour dough. Foaming behaviour of gluten and gluten proteins in urea solutions has been studied by Mita et al. (1977, 1978) and there are several studies of wheat proteins in monolayers (Cockbain and Schulman, 1939; Jaffe and de Coene, 1957; Tschoegl and Alexander, 1960; Yudenfreund, Becher and Brown, 1975). Some physico-chemical aspects of wheat as grain hardness, dough properties, baking behaviour and bread staling have recently been reviewed by MacRitchie (1980).

1.2 The present study

This work is concerned with the colloidal nature of the wheat flour dough and its relation to the baking process. A wheat flour dough contains at least two dispersed phases, air cells and starch granules, which are dispersed in a continuous matrix. The starch granules were not studied explicitly here, which does not mean that the starch granules are considered unimportant. The isolated wheat lipids and proteins were studied both in aqueous bulk phases as well as at the air/water interface. This yields information on the nature of both the air/continuous matrix interfaces and the continuous matrix. Rheological studies on dough were performed to give information which may be used to describe its mechanical behaviour. The mechanical behaviour in turn may be related to the colloidal structure of the dough.

2 MICRO-STRUCTURE AND CHEMICAL COMPOSITION OF WHEAT KERNELS

2.1 Micro-structure of wheat

It is believed that wheat (*Triticum*) was cultivated by the ancient cultures in Asia Minor eight to nine thousand years ago. The most commonly used bread wheats today belong to *Triticum vulgare*. Bread wheats are classified as spring or winter wheats depending upon the time of sowing. Winter wheats are sown in the fall and harvested in the early summer while spring wheats are

sown in the spring and harvested in the late summer. Other cereals used for bread are rye (*Secale cereale*) and triticale (*Triticale*). This part of the thesis concerns the main raw materials used in this study, both from a micro-structural and a chemical point of view. The presentation will be limited to wheat, and only aspects which are essential for the physico-chemical discussion to follow will be considered.

The wheat kernal consists of three major parts, as indicated in Fig. 1. On a dry weight basis a kernal is made up of $\approx 80\%$ endosperm, $\approx 17\%$ bran (pericarp) and $\approx 3\%$ germ (embryo). The endosperm consists of thin-walled cells, which are variable in size, shape and composition depending on their location. Each cell contains starch granules (lens-shaped or spherical) and protein material which makes up the continuous matrix within the cell. The proteins in a cell are of two types, functional ones located in the cytoplasm and/or in the membranes, and storage proteins. The storage proteins are produced in discrete particles (proteoplasts) (Morton, Palk and Raison,

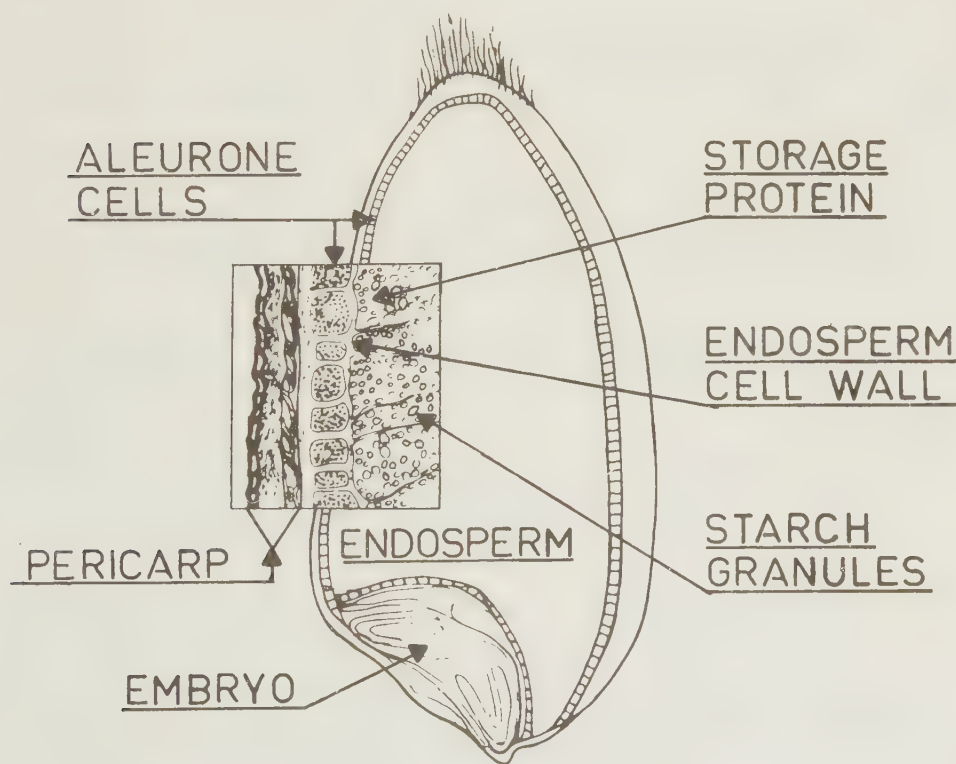


Fig. 1. A schematic drawing of a wheat kernal sectioned longitudinally showing embryo, pericarp, aleurone and endosperm cells.

1964), which disappear as the kernal germinates. The amount of protein in the endosperm increases from the centre outwards. The starch is present as large lens-shaped (lenticular) granules (15-40 μm) and small spherical ones (1-10 μm) (Meredith et al., 1978). There is no sharp distinction in size between the two types of granules. The thickness of the endosperm walls varies between 3 and 7 μm , and the walls contain large quantities of hemi-cellulose. Lipids are distributed in most parts of the cereal kernal both as components of inter- and intracellular membranes, and as spherical minute particles, spherosomes (Barlow et al., 1973; Morton, Palk and Raison, 1964; Seckinger and Wolf, 1967; Simmonds, 1972). The membranes contain mainly polar lipids. In the milling process germ and bran are separated from the endosperm, and the separated parts are milled to fine particles (< 140 μm). If all the milled particles are mixed to a flour with the same weight proportions as in the kernal, the flour has a 100% extraction rate. In normal white baking flour the extraction rate is 60-70%, and this consists of almost only endosperm particles.

2.2 Chemical composition of wheat

The chemical composition of a Manitoba wheat (70% extraction rate) milled in a laboratory is given in Table 1.

TABLE 1
Chemical composition of a Manitoba flour (70% extraction rate)
(McCance et al., 1945)

Flour constituents	%
Ash	0.41
Protein (N x 5.7)	12.9
Protein (N x 5.7) as % of total protein in the wheat	93.6
Oil	1.17
Carbohydrate (as starch)	70.9

As the present study concerns amphiphilic molecules from wheat, a quantitative picture of the different lipid and protein compounds found in wheat flour is of interest. The lipid composition (starch and non-starch lipids) of a composite spring-flour mixture (73% extraction rate) is given in Table 2.

TABLE 2

Composition of non-starch lipids (first extraction with water saturated butanol (WSB) at 20°C) and starch lipids (second extraction with water saturated butanol (WSB) at 90°C) (mg lipid/100 g dry flour) (Morrison et al., 1975).

Lipid	Total	Non-starch	Starch
Sterolester (SE)	90	72	18
Triglyceride (TG)	709	674	35
Diglyceride (DG)	92	86	6
Free fatty acid (FFA)	129	110	19
Esterified monogalactosyl diglyceride (EMGDG) and monoglyceride (MG)	73	66	7
Esterified steryl glycoside (ESG)	77	71	6
Monogalactosyl diglyceride (MGDG)	93	87	6
Monogalactosyl monoglyceride (MGMG)	30	23	7
Digalactosyl diglyceride (DGDG)	226	214	12
Digalactosyl monoglyceride (DGMG)	83	58	25
N-acyl phosphatidyl ethanolamine (APE)	72	72	-
N-acyl lysophatidyl ethanolamine (ALPE)	34	34	-
Phosphatidyl ethanolamine (PE) and Phosphatidyl glycerol (PG)	19	13	6
Lysophosphatidyl ethanolamine (LPE) and lysophosphatidyl glycerol (LPG)	69	10	59
Phosphatidyl choline (PC)	104	66	38
Lysophosphatidyl choline (LPC)	693	36	657
Phosphatidyl serine (PS), lysophosphatidyl serine (LPS), phosphatidyl myo-inositol (PI) and lysophosphatidyl myo-inositol (LPI)	35	11	24
Total lipid	2628	1703	925
Total glycolipids (%)	16	23	5
Total phospholipids (%)	39	14	85

The oil content in Table 1 reflects the content of lipids of the non-polar type (petroleum extract of flour). The true lipid content is approximately 2.5% (w/w), as can be seen in Table 2. The fatty acid compositions of some lipid fractions are shown in Table 3.

TABLE 3
Fatty acid composition of wheat lipid fractions
(Morrison, 1978)

Lipid fraction	Fatty acid (%)					
	16:0	16:1	18:0	18:1	18:2	18:3
Non-starch lipids	16-21	-	2	12-13	60-66	4-5
Non-starch glycolipids	12-14	-	1-2	8-9	71-74	4-5
Non-starch phospholipids	23-37	-	1	9-10	59-63	2-4

The non-starch lipids make up $\approx 1.7\%$ of the total lipids, about 37% of which are polar and 63% non-polar. Approximately 62% of the polar lipids are glycolipids and 38% are phospholipids. The most abundant lipid in starch is lyso-phosphatidyl choline. The only lipids from wheat which have a net charge at the slightly acid conditions in a dough ($\approx 5.5-6$) are phosphatidyl serine (PS), phosphatidyl inositol (PI) and phosphatidyl ethanolamine (PE). The charged lipids make up only $\approx 2\%$ of the total polar lipids (Morrison et al., 1975).

Wheat storage proteins (WS-proteins) account for about 85% (w/w) of the total amount of proteins in wheat flour. These proteins were divided into gliadins and glutenins by Osborne (1907), of which the gliadins represent the water/ethanol soluble fraction and the glutenins the acetic acid (pH 3) soluble part of the remaining fraction, respectively. These two fractions do not represent well-defined proteins but are rather complex mixtures of proteins, and the distribution of these proteins in a solvent will depend on temperature and mixing conditions. This variation, which is greatest for the glutenin fraction, has been studied by Bietz et al. (1975). The crude gliadin fraction, which is extracted by aqueous ethanol, can be separated further into α -, β -, γ - and ω -gliadins by gel filtration (Jones et al., 1963; Meredith and Wren, 1966), or by ion chromatography (Woychik et al., 1960; Huebner and Wall, 1966). The nomenclature for the gliadin protein components is based on their relative electrophoretic mobility in aluminium lactate buffer at pH 3.2 (Woychik et al., 1961). The amino acid compositions of some protein fractions and of purified gliadin proteins are shown in Tables 4 and 5, respectively.

TABLE 4
Amino acid compositions (mol-%) of wheat storage
protein fractions

Amino acids		Gluten ^a	Glutenin ^b	Gliadin ^b
Basic	Lys	1.1	1.5	0.6
	Arg	2.3	2.3	1.8
	His	1.8	1.5	1.8
		(5.2)	(5.3)	(4.2)
Acidic	Glx	34.0	32.4	37.1
	Asx	2.6	2.7	2.4
		(36.6)	(35.1)	(39.5)
Neutral	Gly	5.5	9.1	2.9
	Ser	4.7	5.8	4.5
	Thr	2.5	3.0	2.1
	Tyr	2.3	2.9	1.9
	Cys	1.6	1.2	1.2
		(16.6)	(22.0)	(12.6)
Hydrophobic	Ala	3.5	4.0	2.9
	Val	5.3	4.8	5.1
	Leu	6.9	6.7	7.3
	Ile	3.9	3.3	4.4
	Pro	16.1	13.3	17.4
	Phe	3.8	3.2	4.5
	Trp	0.7	0.9	0.6
	Met	1.4	1.4	1.4
		(41.6)	(37.6)	(43.6)

a) Wu and Dimler, 1963a; b) Wu and Dimler, 1963b.

It is evident from these tables that the WS-proteins contain high amounts of glutamic acid and hydrophobic amino acids (particularly proline) and have a low content of basic amino acids. Glutamic acid is present mainly as glutamine in the protein as is evident from the high amide content, which in most cases equals the content of glutamic acid. As a consequence there are few free carboxyl groups as determined from titration curves (Wu and Dimler, 1963a,b).

Molecular weights for some gliadin components determined from sedimentation equilibrium data (Sexson et al., 1978) are shown in Table 6.

TABLE 5
Amino acid composition of purified gliadin components

Amino acids	A ₂ gliadin ^a	α ₈ ^b	β _{5,6} ^c	γ ₁ ^d	γ ₃ ^d	ω ₂₋₁ ^e
Lys	0.4	0.9	0.2	trace	0.6	0.3
Arg	1.7	1.8	2.3	1.4	1.2	0.6
His	2.1	1.8	2.3	1.5	1.4	0.6
Glx	39.3	38.7	39.6	39.9	39.1	38.8
Asx	2.9	2.8	2.6	2.9	1.7	0.6
Gly	2.5	2.8	1.9	2.4	2.7	1.1
Ser	5.4	5.5	5.3	5.4	4.4	5.9
Thr	1.5	1.8	1.7	1.9	2.2	1.7
Tyr	3.1	2.8	2.5	2.8	0.4	1.2
Cys	1.9	1.8	1.8	2.1	1.0	0
Ala	2.7	2.8	3.5	3.4	3.3	0.6
Val	4.5	4.6	4.0	4.4	4.1	0.4
Leu	8.1	8.3	7.9	7.1	6.3	4.1
Ile	4.5	4.6	3.8	4.7	4.5	1.8
Pro	14.9	15.7	15.8	15.3	18.7	33.5
Phe	3.6	4.3	3.7	3.7	5.6	8.3
Trp	0.4	0	0.1	0.2	0.8	0.3
Met	1.0	0.9	1.1	0.8	1.1	0

a) Platt and Kasarda, 1971

b) Sexson et al., 1978

c) Bietz et al., 1977

d) Huebner et al., 1967

e) Booth and Ewart, 1969

TABLE 6
Apparent molecular weights of isolated gliadin components. The protein concentration used was approximately 0.05% (w/w).

Solvent	pH	ω ₂₋₁ ^a	γ ₁ ^b	γ ₂ ^c	γ ₃ ^b	β _b ^c	α ₇ ^c	α ₈ ^c	Gliadin ^b
0.0167 M aluminium lactate	3.1		34200						46000
3 M urea + 0.15 M KCl	6.3		41900		43600				
3 M urea + 0.15 M KCl	3.1		30300	39800	34700	33000	37100	36900	30300
6 M GuHCl			33600	34600	41000	34200	30400	37500	
1 M Methylformamide	3.4	75500							

a) Booth and Ewart, 1969; b) Sexson and Wu, 1972; c) Sexson et al., 1978

The differences of the apparent molecular weights (MW_{app}) obtained in different solvents have been interpreted (Sexson et al., 1978; Sexson and Wu, 1972) as due to protein aggregation. It should be mentioned that the MW_{app} determined by SDS-PAGE usually are higher than the minimum values of MW_{app} as determined by equilibrium sedimentation analysis (Hamaizu et al., 1974).

The best characterized wheat storage protein to date is α -gliadin (Bernadin et al., 1967). α -Gliadin from certain wheat varieties has been called A-gliadin due to its aggregation behaviour (Platt et al, 1974). It is not a single homogeneous protein, but contains at least four very similar components (Platt and Kasarda, 1971). A-gliadin aggregates in a reversible manner, being aggregated at pH and ionic strength values above 5 and 0.005, respectively (Kasarda et al, 1967). From transmission electron micrographs of A-gliadin Kasarda et al. (1967) suggested that the aggregates are fibrillar in nature with a diameter of around 75 Å. The rheological behaviour of aggregates of A-gliadin in aqueous media has been studied by Bernadin (1975, 1979). He also observed optical birefringence of these aggregates. The binding of the fluorescent hydrophobic probe, TNS (2-p-toluidinylnaphtalene-6-sulfonate) to A-gliadin was reported by Greene and Kasarda (1971).

The N-terminal amino acid sequence of A_2 -gliadin is H_2N - VAL - ARG - VAL - PRO - VAL - PRO - GLN - LEU - GLN - ASN - PRO - SER - GLN - GLN - GLN - GLN - PRO - GLN - GLU - GLN - VAL - PRO - LEU - VAL (Kasarda et al., 1974). Bietz et al. (1977) determined the N-terminal amino acid sequence of α_8 , α_{10} , α_{11} , α_2 , β_5 and γ_1 -gliadin and the sequence turned out to be the same as for A_2 -gliadin. The N-terminal amino acid sequence for γ_2^- , γ_3^- (Bietz et al., 1977) and ω - (Shewry et al., 1980) gliadins are different from that of A_2 -gliadin.

Viscosity measurements have been used frequently to characterize wheat proteins and viscosity data of some protein fractions are listed in Table 7. The intrinsic viscosity $[\eta]$ of a macromolecule depends on its shape (v) and specific volume (v) via the wellknown relation $[\eta] = v \cdot v$. The viscosity factor (v) is 2.5 for spherical particles and any deviation from sphericity leads to higher values of v . As v is about 0.7-0.8 ml/g for most proteins, the data in Table 7 indicate a highly asymmetric shape of the WS-proteins.

TABLE 7

Intrinsic viscosities of wheat storage protein fractions in aluminium lactate buffer, pH 3.1

Preparation	[η] ml/mg
Glutenin	77 ^a
Glutenin (reduced and alkylated)	23 ^a
Gliadin	15 ^b
Gliadin (reduced and alkylated)	18 ^b
Purified gliadin	9.3 ^c

a) Beckwith and Wall, 1966; b) Nielsen et al., 1962;

c) Beckwith et al., 1966

The apparent molecular weights of the WS-proteins in Table 6 indicate that these proteins are not in a monomer state in aluminium lactate buffer and, therefore, the high intrinsic viscosities probably reflect an asymmetrical shape of the protein aggregates.

The amount of gliadin in the WS-proteins is between 30% and 45% of the total protein (Wall, 1979). The differences in gliadin content recorded in various investigations are probably due to variations in extraction conditions and the methods of characterization of these proteins. Danno et al. (1974) used 0.5% sodium dodecyl sulfate (SDS) to extract wheat flour proteins. They found that approximately 75% of the flour nitrogen was solubilized into a clear solution, and this figure did not vary significantly between different flours. From electrophoresis and molecular sieve chromatography they concluded that the proteins soluble in SDS were gliadin-like, mainly consisting of components with a MW_{app} less than 75000. If it is assumed that all non-WS-proteins (around 15%) are also extracted by SDS the gliadin content of the WS-protein is nearly 83%. The proteins that were insoluble in SDS were dissolved completely by the addition of 2-mercaptoethanol. These proteins contained the glutenin fraction (Danno et al., 1976). Later Danno et al. (1978) fractionated and characterized these SDS-insoluble WS-proteins. They found 4 fractions one of which did not have the characteristic amino acid composition of a WS-protein. 55% of these polypeptides with WS-protein characteristics consisted of gliadin-like components, as judged by the amino acid composition, while 35% consisted of components characteristic of WS-proteins with MW_{app} (SDS-PAGE) different from the gliadins. From the results of Danno and co-

workers, a more precise physico-chemical definition of the different protein components related to gliadin and glutenin can be performed (Table 8).

TABLE 8
Chemical and physical characteristics of gliadin
and glutenin polypeptides

Characteristics	Gliadin	Glutenin
Isoelectric point	8.1-6.5	6.5-4.2
MW _{app} (SDS-PAGE)	< 75000	> 75000
Amount of Phe (mol%)	3.5-9	< 35
Amount of Gly+Tyr (mol%)	< 5.8	> 5.8
Amount of Glu (mol%)	> 35	> 35
Flour nitrogen (%)	≈ 75	≈ 8
WS-protein nitrogen (%)	≈ 90	≈ 10

3 WHEAT LIPIDS AND PROTEINS IN AQUEOUS BULK PHASE AND AT THE AIR/WATER INTERFACE

The non-carbohydrate material of wheat flour consists mainly of lipids and proteins. These are of particular interest in this study, since they are surface active and aggregate in water. Thus, the behaviour of wheat lipids and proteins in aqueous bulk and at the air/water interface is essential when the colloidal nature of the dough is considered.

3.1 Surface behaviour

3.1.1 Wheat lipids

The monolayer characteristics of many polar lipids of biological origin are known. The monolayers of phosphatidyl cholines (PC) at the air/water interface are particularly well characterized (Phillips and Chapman, 1968; Tabak et al., 1977). The general monolayer features of polar lipids at a temperature above the chain melting temperature are characterized by an isotherm of the so-called liquid-expanded type, with a cross-sectional area of about $30 \text{ \AA}^2$ per acyl chain at the collapse point. This is well illustrated in Fig. 2, which shows the surface pressure-area (π -A) isotherm for digalactosyl diglyceride (DGDG). The preparation of the DGDG sample and the monolayer technique used are described in (I) and (IV), respectively. The area and the surface pressure at the collapse are $61 \text{ \AA}^2/\text{mol}$ and 40 mM/m , respectively.

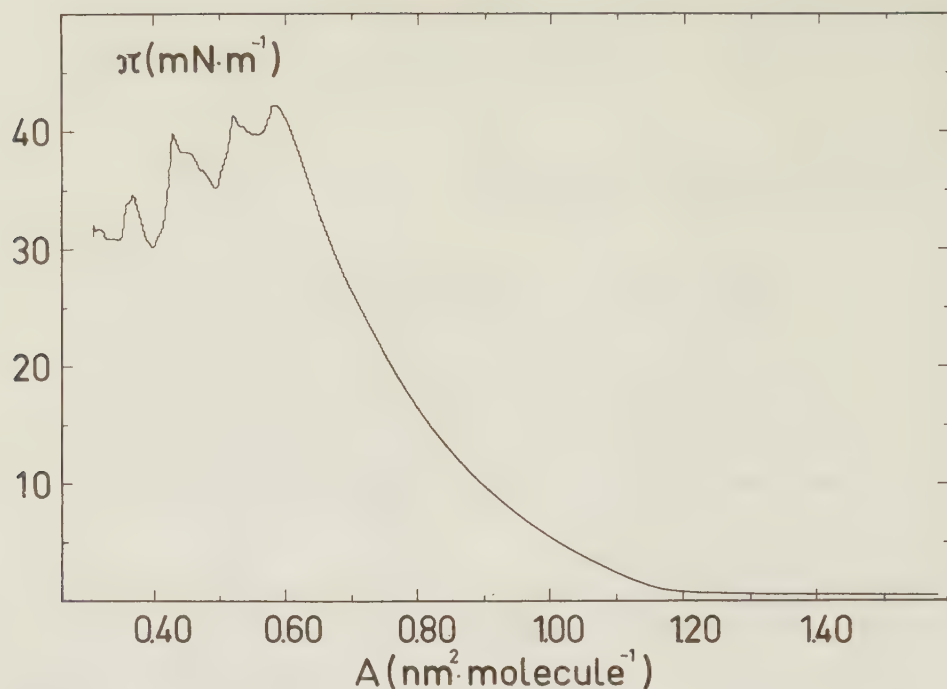


Fig. 2. Π -A isotherm of digalactosyl diglyceride spread from chloroform/methanol/water on double distilled water at 23°C. The compression velocity is 0.117 nm²/min.

The observed equilibrium spreading pressure (ESP) for DGDG is equal to its collapse pressure. This is also to be expected since the monolayer at this surface pressure is in equilibrium with the stable bulk phase of DGDG, which is a lamellar mesophase (Shipley et al., 1973). The Π -A isotherm observed here is very similar to the isotherms for DGDG and monogalactosyl diglyceride (MGDG), which are reported by Oldani et al. (1975). The ripples observed in the Π -A isotherm after collapse (Fig. 2) are caused by the overflow of collapsed monolayer material from the surface balance trough, when the monolayer material carries with it water. A phosphatidyl choline (PC) monolayer exhibits the same behaviour when it is compressed past its collapse point.

3.1.2 Wheat storage proteins

The monolayer of A-gliadin at the air/water interface is described in (IV). This is the first report on the monolayer behaviour of a wheat storage protein with chemical and physical properties known to some extent. The A-gliadin molecules behave like simple amphiphiles at the air/water interface with regard to its stability, and the Π -A isotherm exhibits both a phase transi-

tion and a well developed collapse. The phase transition observed for A-gliadin, which is of the first order, has been interpreted as the point where the individual molecules condense into a coherent monolayer. The ESP observed for A-gliadin equals the collapse pressure of the monolayer. This observation, together with the observation on the stable aqueous bulk phase of A-gliadin (discussed on page 33), is the basis for the proposal that the monolayer is in equilibrium with a lamellar aqueous mesophase after the collapse point. The general monolayer characteristics of A-gliadin is similar to those of polar lipids. This requires an amphiphilic nature, and therefore the A-gliadin molecules must have a polar/non-polar asymmetry on its surface.

Crude wheat gluten (Tschoegl and Alexander, 1960) and unfractionated gliadins (Cockbain and Schulman, 1939; Jaffe and de Coene, 1957; Yudenfreund et al., 1975) have been studied as monolayers at the air/water interface. The π -A isotherms reported for both gluten and gliadin are very similar to the isotherms observed for many other proteins (Bull, 1947), showing a S-shaped isotherm with a 'limiting area' of around $1 \text{ m}^2/\text{mg}$. The chemical analysis of WS-proteins in general, and of gliadin in particular, indicates that the major difference between these proteins is their molecular weight and that their primary structures are very similar. One would therefore expect that the monolayer behaviour of each of these proteins in a monodisperse state would be similar to each other, and thus similar to that of A-gliadin discussed above.

3.1.3 Wheat lipid and protein

The molecular interactions between proteins and lipids can be studied conveniently using the surface balance, particularly if both constituents can be spread from the same solvent. Mixtures of A-gliadin and DGDG were studied at the air/water interface using the monolayer technique described in (IV) with chloroform/methanol/water as solvent. Mixtures of A-gliadin and DGDG in the approximate molar ratios 1:1 and 1:35 were used (assuming molecular weights of 920 and 32000 for DGDG and A-gliadin, respectively). The π -A isotherms of DGDG and A-gliadin are shown in Fig. 2 and (IV, Fig. 1), respectively, and those of the two mixtures are shown in Figs. 3 and 4. For mixed monolayers, in which the components are immiscible, the following relation should be fulfilled:

$$A_{12} = N_1 A_1 + N_2 A_2 \quad (1)$$

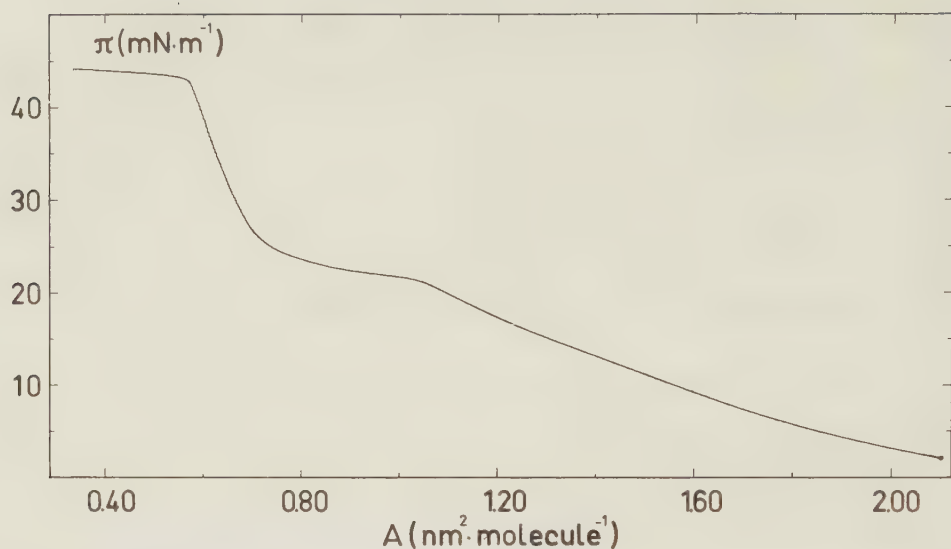


Fig. 3. Π -A isotherm of digalactosyl diglyceride and A-gliadin monolayer spread from chloroform/methanol/water on double distilled water at 23°C. 2.74 mol-% of A-gliadin is used. The compression velocity is 0.117 nm²/min.

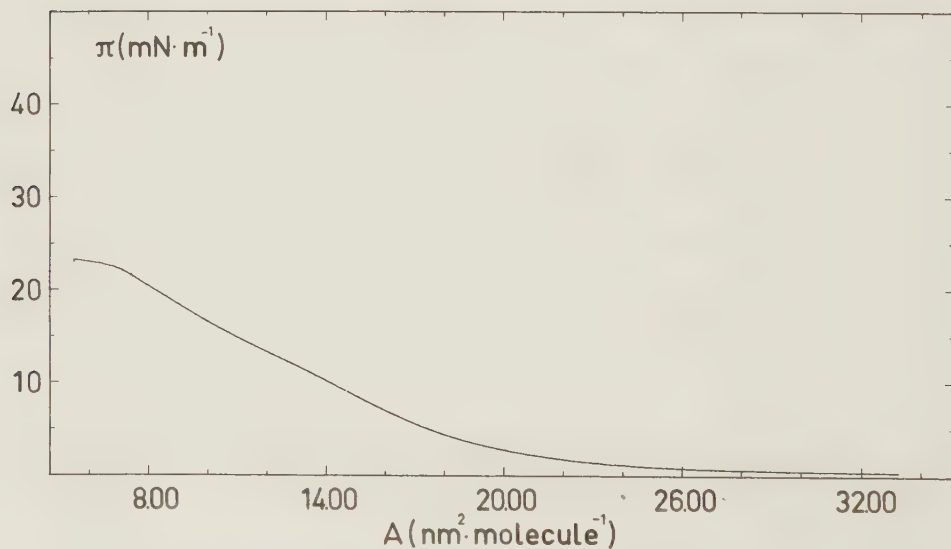


Fig. 4. Π -A isotherm of digalactosyl diglyceride and A-gliadin monolayer spread from chloroform/methanol/water on double distilled water at 23°C. 46.98 mol-% of A-gliadin is used. The compression velocity is 0.117 nm²/min.

where A_{12} is the average molecular area of the mixed film, N_1 and N_2 the mole fractions of the components, and A_1 and A_2 the molecular areas of the two single component films at the same surface pressure (Crisp, 1949a). From the surface phase rule (Crisp, 1949b) follows that for a mixed film of immiscible components the component with the lower ESP will be squeezed out of the monolayer at its ESP, regardless of the initial proportion of the two components. This is observed in Figs. 3 and 4, where the A-gliadin molecules are displaced from the monolayer at the collapse pressure for A-gliadin. All four isotherms are shown in Fig. 5 together with the values calculated from equation (1). It is clear that A-gliadin and DGDG do not associate in the molecular films. This is also the behaviour to be expected since lipid and protein molecules rarely associate unless they carry opposite net charges (Rand, 1976).

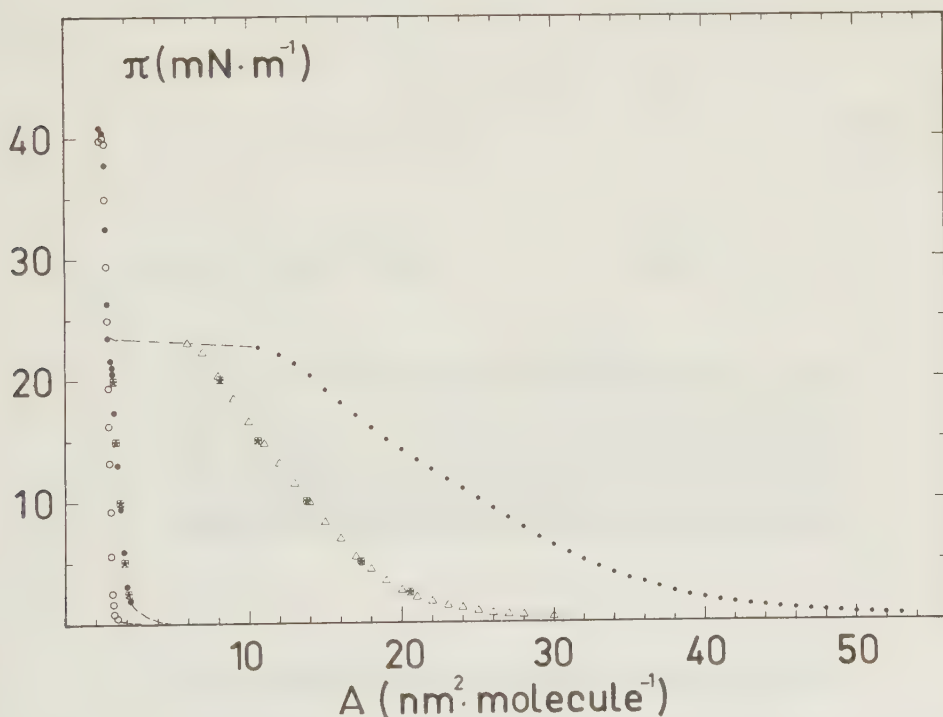


Fig. 5. Collected Π -A isotherms for A-gliadin (●) from (IV), DGDG (○) (Fig. 2) and mixtures (△) (Fig. 4) and (●) (Fig. 3). $\boxtimes$ are calculated values from equation (1).

It is interesting to observe the absence of 'ripples' after collapse in the isotherm shown in Fig. 3. This indicates that water is prevented to associate with the collapsed lipid monolayer. A possible explanation for this is that the collapsed A-gliadin monolayer is forced into the aqueous phase, and that this monolayer interacts with the lipid monolayer. Such an association is schematically shown in Fig. 6. Thus, this association should not be regarded as an interaction between individual molecules but as an interaction between two phases.

3.2 Aqueous bulk behaviour

3.2.1 Wheat lipids

Before the behaviour in aqueous media of the total wheat lipids is discussed, it may be useful to summarize the known behaviour of the major individual lipid components DGDG, MGDG and phospholipids (dominated by PC). At water ratios above a few percent the liquid crystalline phase both of PC (Small, 1967; Chapman et al., 1967) and DGDG (Shipley et al., 1973) at 20°C is the lamellar phase. MGDG (Shipley et al., 1973) forms only a reversed hexagonal liquid-

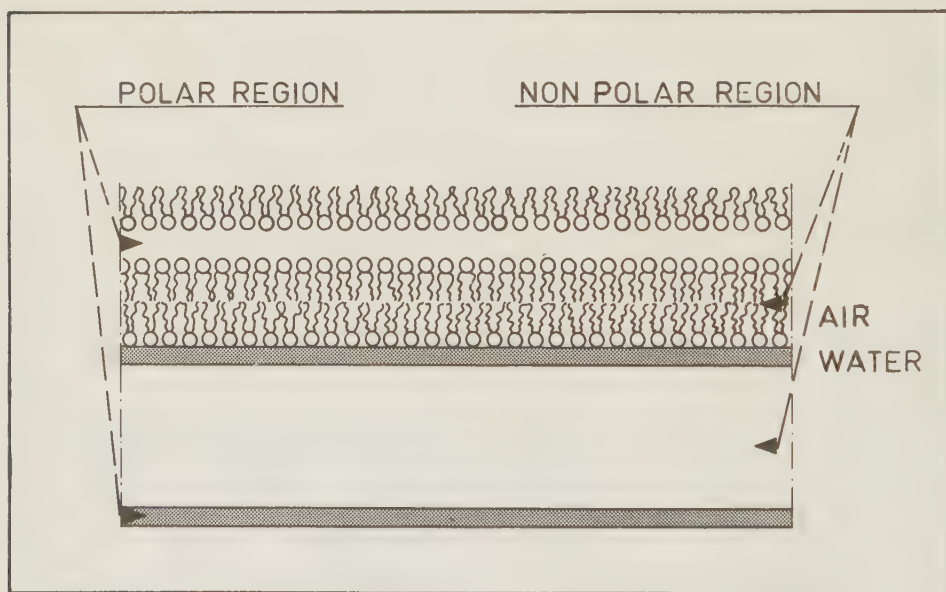


Fig. 6. Schematic illustration of collapsed monolayers of DGDG and A-gliadin at the air/water interface. The lipid molecules are illustrated with a polar head (○) and a hydrocarbon chain tail. The polar regions of the aqueous A-gliadin phase are illustrated as dark bands. These regions consist of both water and the polar moieties from the protein molecules.

-crystalline phase at 20°C. All the major non-starch polar lipids thus behave as polar lipids, which swell in water but do not form micellar solutions. The behaviour in aqueous media of mixtures of DGDG and MGDG has been described (Larsson and Puang-Ngern, 1979). They occur in a weight ratio of 3:7 in wheat lipids (Morrison et al., 1975), and at this ratio only the lamellar liquid-crystalline phase exists (Larsson and Puang-Ngern, 1979).

The aqueous phase behaviours of more complex polar lipid mixtures like brain lipids and mitochondrial lipids have been examined by Luzzati and Husson (1962) and Gulik-Krzywicki et al. (1967). Both the brain (50% PE, 35% PC, 13% PI) and the mitochondrial (29% PE, 35% PC, 10% PI, 20% cardiolipids) lipids form a reversed hexagonal liquid-crystalline phase at low water content above 20°C. Thus complex mixtures of polar lipids behave like a single component with respect to the phase rule. The phase behaviour of a three component system, e.g. if the lipid mixture also contains non-polar lipids, is conveniently described in a ternary phase diagram.

The aqueous and salt-aqueous phase behaviours of wheat lipids have been studied at 23°C and described in ternary phase diagrams (I,II). The aqueous phase diagram for lipids extracted from wheat flour (Amy) is shown in (I, Fig. 2). It is dominated by two types of phases; liquid-crystalline phases and the liquid phases. The isotropic phases are oil, water and a L2-phase. The latter is proposed to be an inverse micellar phase (Ekwall, 1963). The liquid-crystalline phases found are of the reversed hexagonal and lamellar type. The optical textures of these two phases are illustrated in Fig. 7. The transition from reversed hexagonal to lamellar liquid-crystalline phase in this system occurs at about 20% water, which is very similar to the value found for both brain and mitochondrial lipids (Luzzati and Husson, 1962; Gulik-Krzywicki et al., 1967). A L2-phase was observed in the water-rich corner of the ternary system. Its structure was proposed (I) to be related to the lamellar liquid-crystalline phase. This is the first reported observation of an aqueous L2-phase formed by naturally occurring lipids. The exact extension of this L2-phase, particularly towards the water/polar lipid axis, is difficult to measure experimentally. We know, however, that the L2-phase does not form in a binary system of water and the most polar fraction of wheat lipids. It is evident from (I, Fig. 2) that if the wheat lipid/water system is represented as a ternary one, it deviates from the phase rule of a three-component system, as this rule limits the components to three, in a phase area of a three component system. From the two-phase region with L2 and lamel-

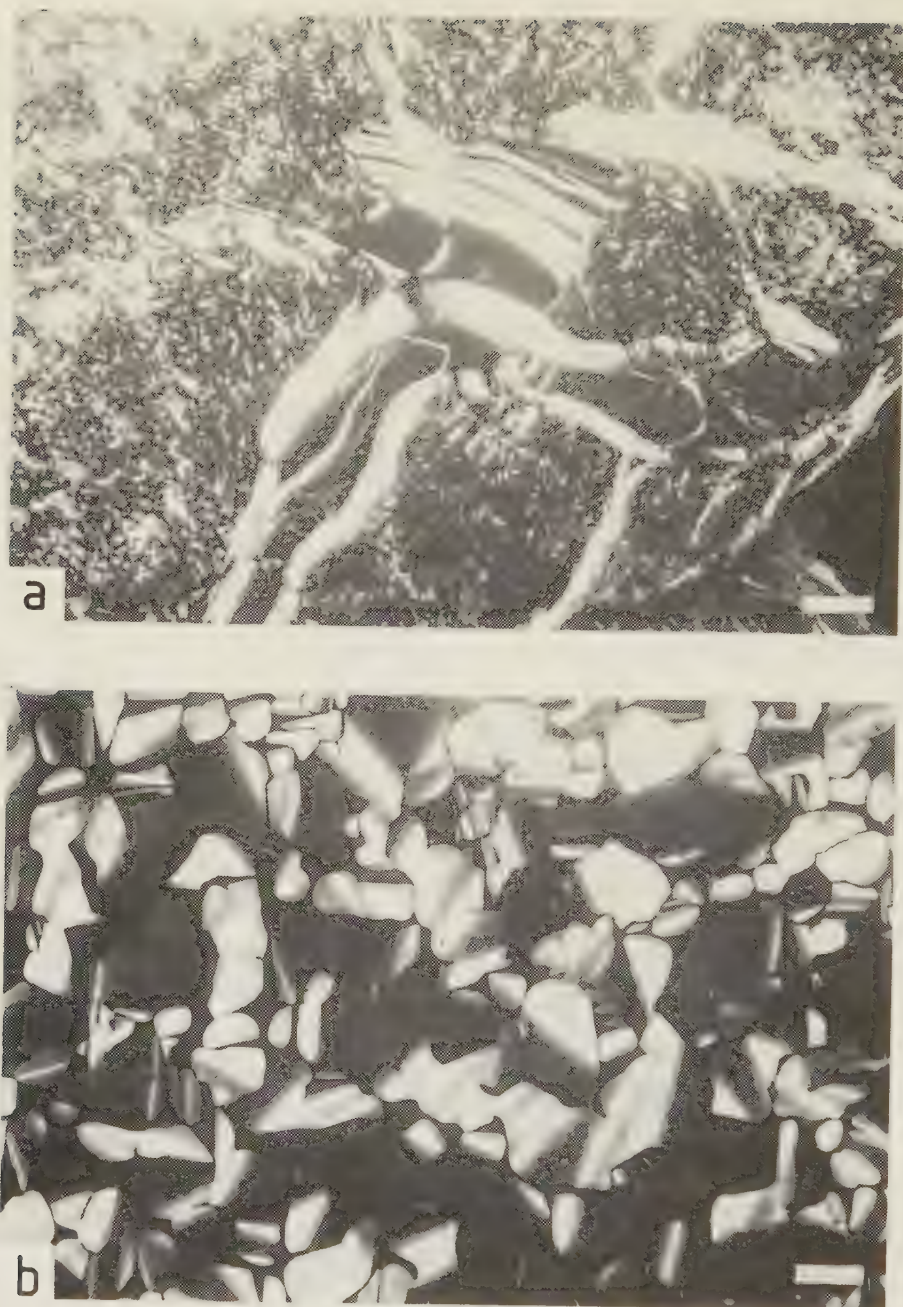


Fig. 7. Photomicrographs in polarized light of wheat lipids/water system. Bar 40 μm . a) Lamellar liquid-crystalline phase, b) reversed hexagonal liquid-crystalline phase.

lar liquid-crystalline phases a four-phase region extends up to the oil corner. This deviation from the phase rule is probably due to a segregation within the polar lipids, i.e. the polar lipids do not behave as one component. This anomalous behaviour is not seen when aqueous solutions of salts are used to construct the phase diagram (II, Fig. 4). Under these conditions always a small amount of hexagonal phases was present. It is likely that this salt-induced segregation involves the formation of a hexagonal phase from negatively charged lipids.

It is interesting to compare the wheat lipid/water system with a simple ternary amphiphile/water system, such as 1-monocaprylin/decanol/water (Fig. 8).

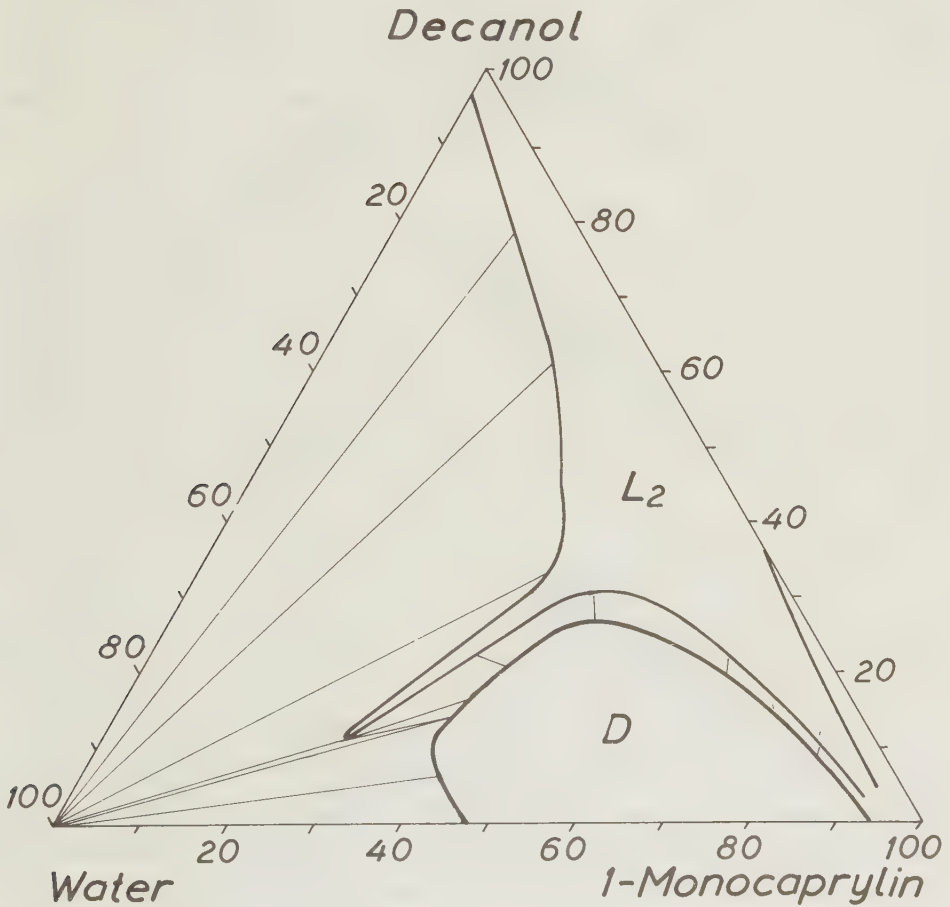


Fig. 8. Ternary phase diagram of 1-monocaprylin/decanol/water at 20°C (Ekwall et al., 1969). D is the lamellar liquid-crystalline phase.

The behaviour of the two systems near the water/polar lipid axis is the same, except for the presence of the hexagonal phase in the wheat lipid/water system. The most significant difference between these two systems is, however, the extension of the L2-phase. This phase only exists as an isolated island in the water-rich corner of the wheat lipid/water system, while it extends as a triangle with its base on the 1-monocaprylin/decanol axis and the top pointing to the water corner in the 1-monocaprylin/decanol/water system. The L1-(micellar solution) and L2-phases can in some systems successively transform into each other, so that one liquid phase extends over the whole diagram. Ekwall and Mandell (1969) showed that in sodium caprylate/water/caprylic acid, the L2-phase became discontinuous in the water-rich corner when temperature was increased. The same principal behaviour of a discontinuous L2-phase has been observed in the tetraglycerol monoauric/water/soybean oil system (Pilman et al., 1980). The extension of the L2-phase region near the non-polar/polar lipid corner in the wheat lipid system is difficult to determine. The extension of this phase at different temperatures can be illustrated as in Fig. 9. The L2-region has a bridge-like extension in relation to the temperature.

Even considering the deviation of the wheat lipid/water system from an ideal three component system, it is remarkable how principally similar the former is to simple amphiphile/water systems. This is an extension of earlier observations, where mixtures of polar membrane lipids were shown to behave like one component. The introduction of a complex non-polar lipid mixture thus gives a phase diagram similar to that of a simple ternary system.

The structure of the L2-phase observed here is discussed in (I). There exists little general structural information on the L2-phase so far. From X-ray diffraction measurements Larsson (1972, 1979) has described the L2-phase in monoglyceride/water systems as structurally related to the mesophase obtained when the L2-phase is cooled. This model of the L2-phase, which is related to the lamellar mesophase, is illustrated in Fig. 10 for the wheat lipid/water system. The motivation for adopting this model (I) is illustrated in Fig. 11. In this figure a L2-phase is viewed in a polarizing microscope when water is evaporating. The strongly birefringent border on the left side is the outer edge of the drop. The L2-phase is seen as the black isotropic area. As the water evaporates, particles with an optical texture resembling that of liposomes are formed in the L2-phase. Worm-like aggregates with a two-fold symmetry, similar to so-called 'battonnets' are also observed. The optical texture

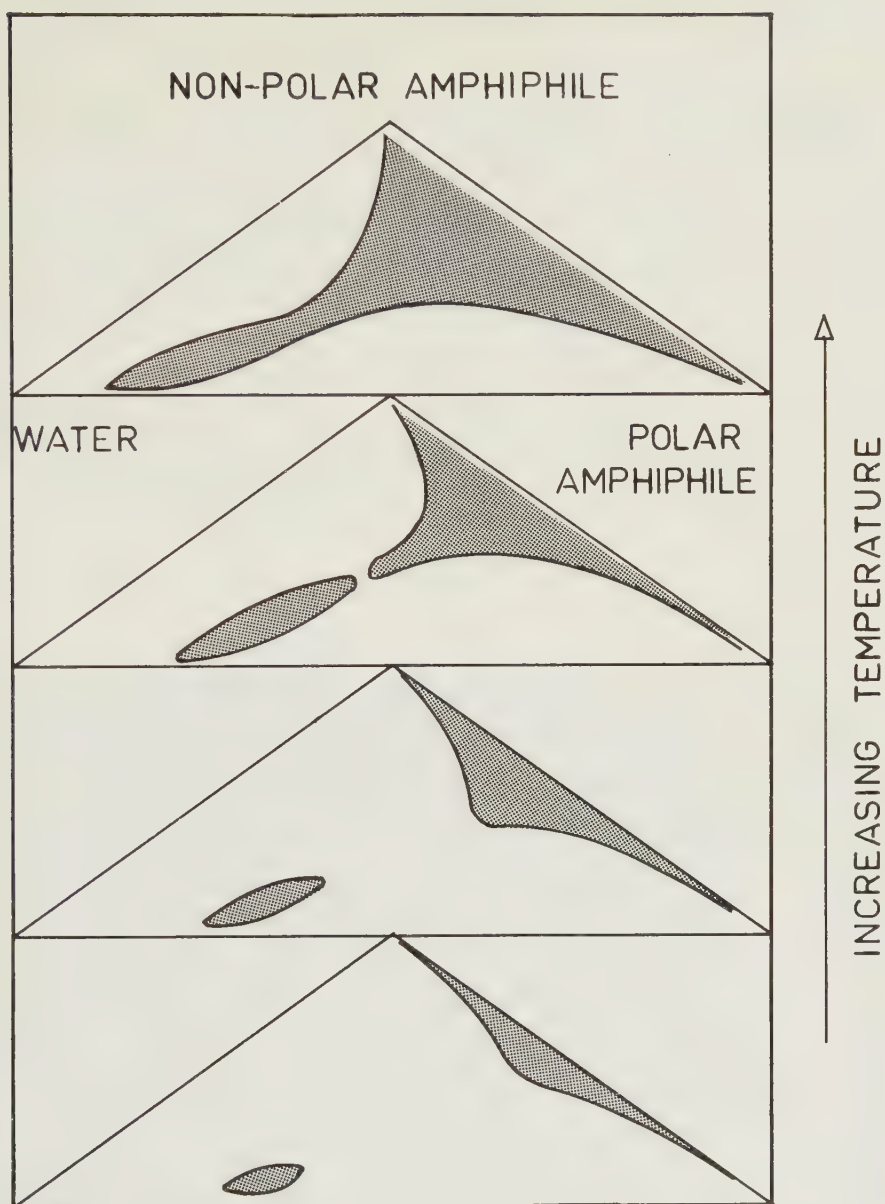


Fig. 9. Schematic illustration of the L2-regions (dark areas) in a ternary system (polar amphiphile/water/non-polar amphiphile) as a function of temperature



Fig. 10. Proposed structure of the L2-phase in the wheat lipid/water system. Dark areas = water regions. (O) polar head group. Triglycerides are indicated in the continuous medium.

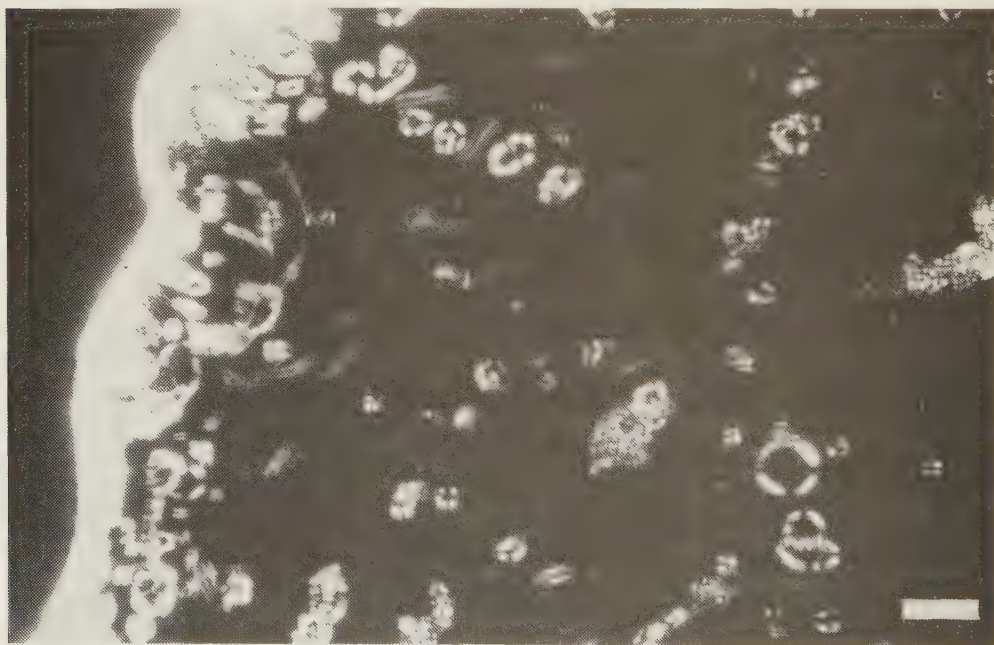


Fig. 11. Photomicrograph (polarized light) of a L2-phase (wheat lipids and water) as observed when water is evaporating from the sample. The birefringent border to the left is the lamellar mesophase and the closed concentric birefringent particles are 'hydrophobic liposomes' dispersed in the L2-phase. Bar 40 μm .

of the material at the edge is that of a lamellar mesophase. The structures formed as the water evaporates are expected to have the hydrophobic hydrocarbon tails facing the oily continuous phase. The proposed structure of such a 'hydrophobic' liposome is illustrated in Fig. 12.

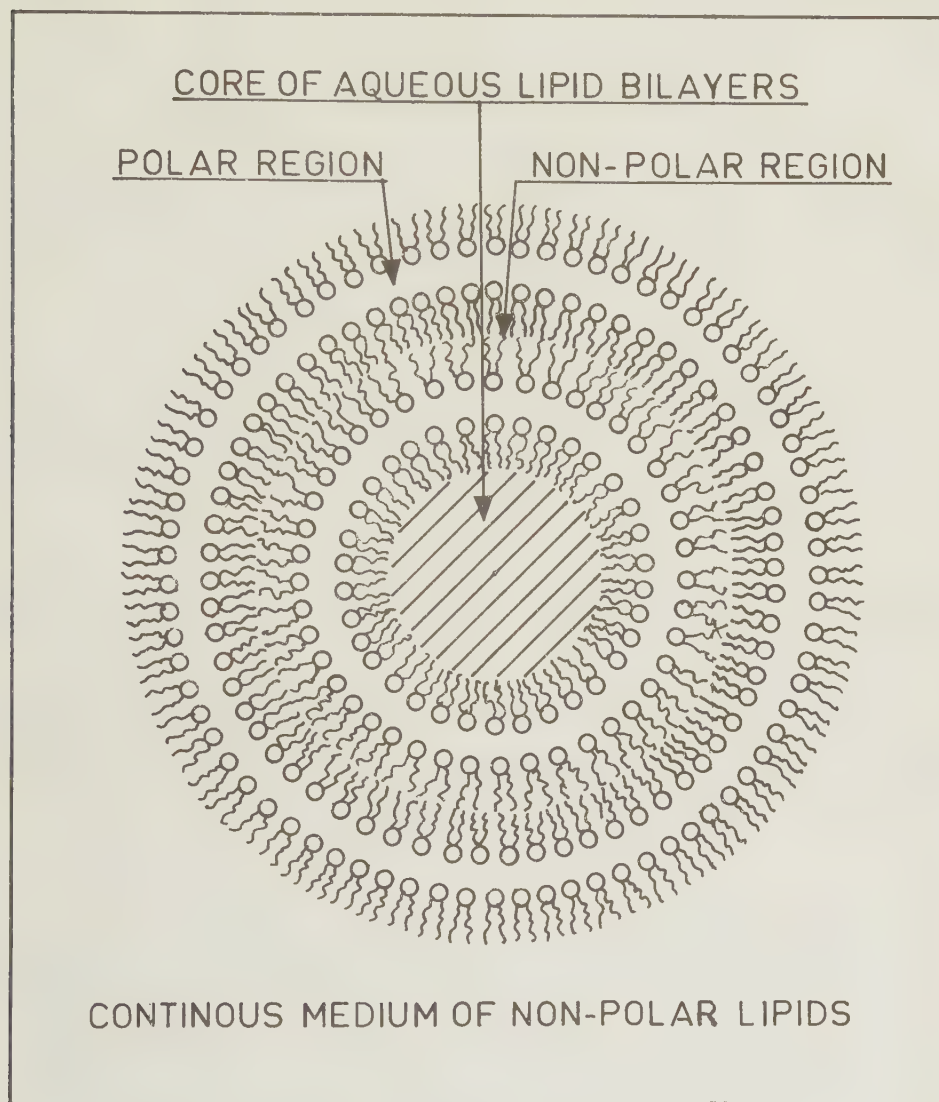


Fig. 12. Schematic picture of a particle with lamellar liquid-crystalline structure which is dispersed in a non-polar lipid. The polar lipid molecules are illustrated as in Fig. 6.

3.2.2 Lipids from different wheat cultivars and from rye and triticale

In the previous section the aqueous behavior of lipids extracted from the wheat variety Amy was discussed. A similar study has been performed on two other wheats, Starke and Maris Huntsman (III). The lipids extracted from these latter wheats did not behave as simply as those from Amy, and it was difficult to determine the ternary phase diagrams. This difficulty was due to the fact that a hexagonal phase occurred together with the lamellar one up to high water contents and the separation of these phases was difficult. As the amount of oil separating from a lipid sample when it is exposed to water is nearly the same for all wheat varieties examined, a simpler method than the previously described (I,II) of representing the lipid/water phase behaviour was chosen. This is illustrated in (III, Fig. 6) for the wheat varieties Amy and Maris Huntsman, and for rye and triticale. The most significant difference between the wheat cultivars is the extension of the hexagonal and lamellar phases. For Amy the hexagonal phase only exists in the region of low water content, whereas this phase exists up to high water contents for the other two cultivars. The extension of the lamellar phase follows an opposite pattern.

The aqueous phase behaviour of lipids from rye and triticale was also studied (III). Rye and wheat lipids behave very differently. A liquid-crystalline phase was observed for rye lipids up to 20% of water and this transformed into a L2-phase when the water content was increased further. The lipids extracted from triticale behave in a manner which is intermediate between that of rye and wheat. However, the same type of liquid crystalline → L2 phase transition dominates the phase behaviour.

3.2.3 Wheat storage proteins

The nature of wheat storage proteins has challenged scientists since the 18th century when Parmentier (1773) observed that gluten could be largely dissolved in vinegar and partly dissolved in spirits of wine. Many publications concerning the nature of these proteins have appeared since then. The discussion here will focus on the physical state of these proteins, and in particular on their state in the high concentration region.

A-gliadin is believed to be a compactly folded globular protein, which aggregates reversibly into fibrillar structures at pH 5, when the ionic strength is above 0.005 (Kasarda et al., 1967; Bernadin et al., 1967). The aggregation

takes place without any substantial change in the secondary structure (Kasarda et al., 1968). The aggregates formed appeared to be fibrils, with a diameter of about 80 Å according to electron micrographs (Kasarda et al., 1967). Bernadin (1975) observed A-gliadin aggregates which at protein concentration above 5% (w/w) separated into birefringent regions, which were in equilibrium with isotropic solution. The birefringent regions were not homogeneous but biphasic mixtures of two birefringent phases (Bernadin, 1975). They were transformed to stable gels when the solution was sheared in an oscillatory mode. From the temperature dependence of the shear stress, the interaction between A-gliadin fibrils was estimated to be 10.2 Kcal/mol below 35°C and 6.25 Kcal/mol above 35°C (Bernadin, 1978). Bernadin (1975) interpreted the birefringent regions as a dispersion of a lyotropic nematic mesophase in a dilute birefringent phase.

Micrographs of aqueous A-gliadin preparations in water are shown in Fig. 13. The optical texture in these pictures resembles textures of known lamellar mesophases, and the sample appears homogeneous which indicates that only one phase is present. The X-ray diffraction data, showing a broad diffraction line at 28.5 Å, prove the existence of long range order in this aqueous A-gliadin phase. Thus this phase must be considered as a liquid-crystalline phase.

Lyotropic mesophases are known to be formed by two types of polymers:

1. Polymers with a rigid rod-like conformation. Examples of this type of polymers are synthetic homopolypeptides and DNA. Solutions of homopolypeptides, which form α -helical structures, separate with increasing concentrations into birefringent mesophases, which in the case of poly (γ -benzyl-L-glutamate) (Robinson, 1956) and DNA (Robinson, 1961) are believed to be of cholesteric type. Bernal and Fankuchen (1937, 1941) observed that the rod-like tobacco mosaic virus formed an aqueous mesophase.
2. Amphipatic polymers, which possess exposed hydrophilic and hydrophobic parts. Examples of amphipatic polymers are the block copolymers, which yield lyotropic mesophases of the same type as is observed in aqueous lipid systems (Gallot, 1978).

The behaviour of A-gliadin at the air/water interface indicates that this protein is amphipatic in character and, therefore, behaves like an amphipatic copolymer rather than a rigid rod-like polymer.



Fig. 13. Photomicrographs taken in polarized light of the A-gliadin water system. a) Bar 170 μm ; b) Bar 40 μm .

The unusual behaviour of WS-proteins in solution was observed at the beginning of this century by several scientists. Some of these early physico-chemical studies still represent the frontier of knowledge in this area and, therefore, they will be reviewed here. Dill and Alsberg (1925) studied the effects of temperature and ethanol concentration on the solubility of gliadin in aqueous ethanol. They observed that an optically clear gliadin solution made at a certain temperature became turbid upon cooling. The temperature where the solution became turbid was well defined, and it was called the critical peptization temperature (CPT) of the solution. They also observed that the turbidity disappeared upon reheating of the turbid solution. The CPT was at a minimum in a 60% (v/v) ethanol solution (Dill and Alsberg, 1925). The CPT was independent of protein concentration above 25% (w/w) in this solvent. A few years later Haugaard and Johnson (1930) published a comprehensive investigation on the fractionation of gliadin in aqueous ethanol. They used the temperature dependence of the solubility of gliadin to obtain gliadin fractions. Gliadin was dissolved at room temperature and as the temperature was lowered to 0°C the solution separated into two layers. The bottom insoluble layer of gliadin was redissolved at room temperature and again separated at 0°C. The CPT of these separated gliadin fractions was examined as a function of protein concentration, ionic strength, pH, and solvent composition. The separation process was reversible and the insoluble fraction at 0°C was sol-like, highly viscous and optically homogeneous. They described the behaviour of their gliadin preparation as an association/dissociation of two fractions of gliadin with different solubility.

Haugaard and Johnson (1930) also investigated the phase equilibria between gliadin, ethanol and water at 0°C. For this purpose the compositions of the solution and of the separated mass were determined for different mixtures of gliadin, water and ethanol after incubation for two weeks at 25°C. They presented their results as a ternary phase diagram, and this is shown in Fig. 14. The upper curve represents the composition of the separated mass and the lower curve represents the composition in the clear solution. Equilibrium composition data obtained at 20°C for the same system (Fig. 14) have been published by Bungenberg de Jong and Klaar (1932). The equilibrium data of the two investigations presented in Fig. 14 are complementary with respect to the composition range covered. The maximum solubility of gliadin at the optimum 52.2% aqueous ethanol is not known. However, Dill and Alsberg (1925) gave a value of 25% (w/w) in this medium. By combining these data obtained

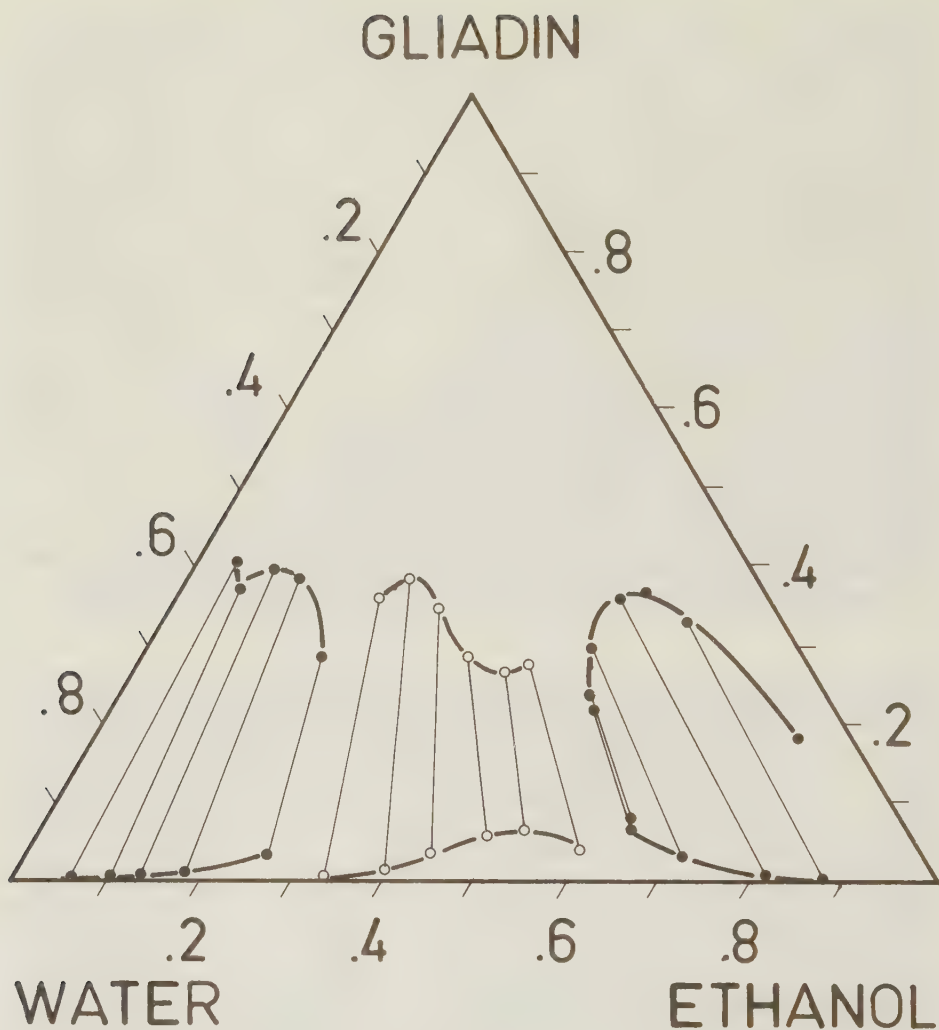


Fig. 14. Ternary phase diagram of ethanol/gliadin/water at 25°C (●) and 0°C (○).

at 0°C and 20°C, a probable shape of the diagram at room temperature can be obtained, since an increase in temperature is likely to shift only the phase border towards each other, at an ethanol/water composition of around 50/50 by weight (Fig. 15).

The structures of the two phases observed in the gliadin/ethanol/water system are unknown. It is evident from Fig. 14 that the relative composition of ethanol and water is constant in the two phases at low ethanol concentrations

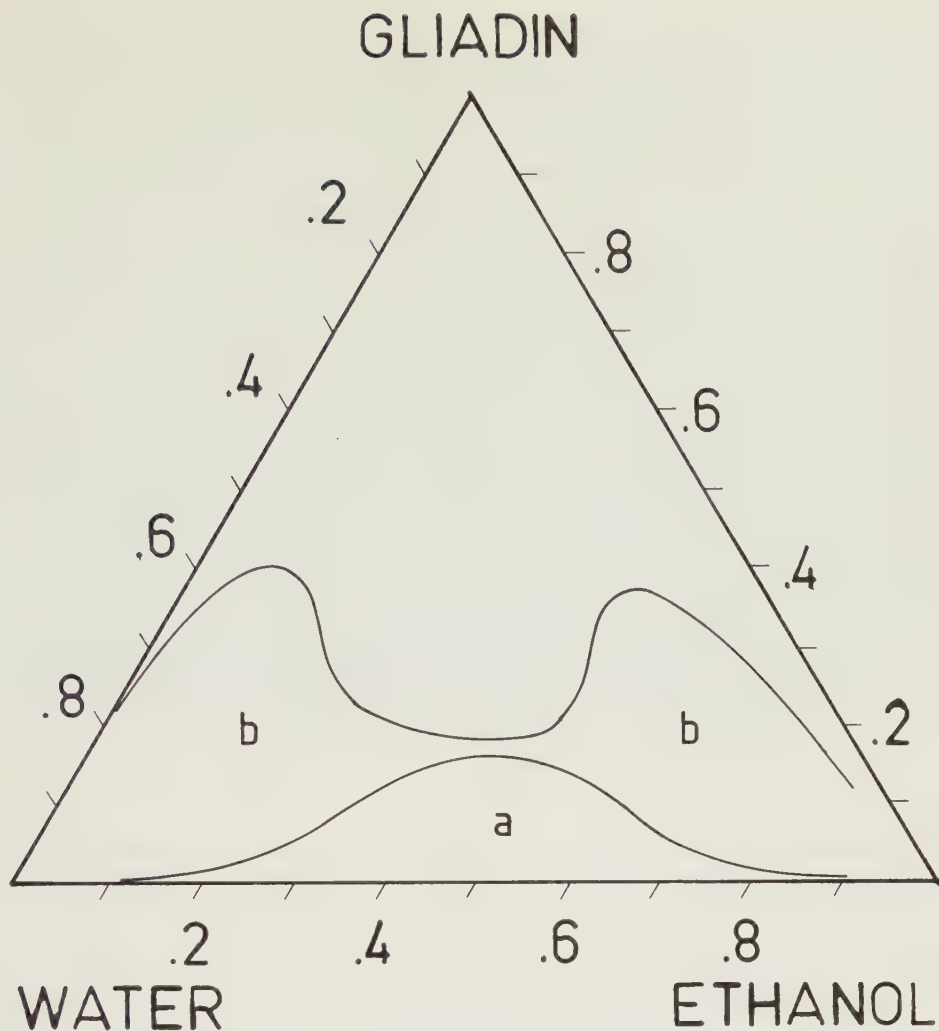


Fig. 15. A possible shape of the phase boundaries in the ternary ethanol/ /gliadin/water system at 25°C. a. One phase area; b. two phase area.

(below 40%). The phase with low protein content was suggested by Haugaard and Johnson (1930) to be a solution. It is uncertain, however, whether this is a true monomer solution or a solution of micellar type. Bungenberg de Jong and Klaar (1929) related the observed cloudiness of the concentrated phase to the presence of spherical liquid droplets. These droplets thus represent a dispersed analogue of the concentrated phase. In a series of investigations Bungenberg de Jong and Klaar (1930a,b, 1931, 1932) studied the formation of these droplets by viscosimetry and turbidimetry. It should be mentioned that ethanol or any other non-aqueous solvent is not required for the formation of these droplets. This is shown in Fig. 16, which displays droplets separa-

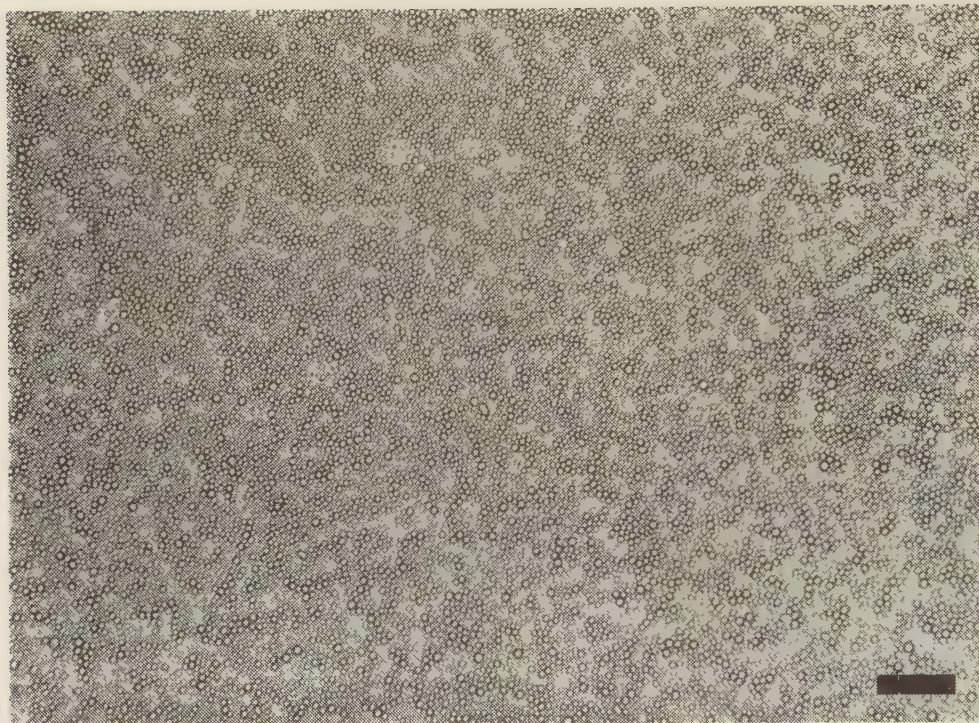


Fig. 16. Photomicrograph (normal light) of wheat storage protein droplets dispersed in water. Bar 72 μm .

ted from an aqueous solution of WS-proteins.

Bungenberg de Jong (1932) describes the colloidal behaviour of gluten in terms of the binary mixture of gliadin and glutenin. His preparations of gliadin and glutenin have isoelectric points of 6.5-6.6 and 5.3-5.4, respectively. He described the turbidity of the mixtures in the pH-range where the two fractions had different net charges. The effect of electrolytes on a given protein mixture at a given pH was also studied and he concluded that gluten was not a physical mixture of gliadin and glutenin but rather an electrostatically interacting system. Although his preparations of gliadin and glutenin were not very well defined, it is clear from more recent studies that glutenins have significant lower values of their isoelectric points than gliadins (Danno et al., 1978).

Aqueous phase separation of proteins, which gives an optical isotropic protein-rich liquid in equilibrium with a dilute protein solution, was called coacervation by Bungenberg de Jong and Kruyt (1930). Coacervation is a reversible, concentration dependent phase separation, which is observed as the

formation of liquid droplets. It may be elicited by changes in temperature, pH, salt concentration, solvent composition or by the addition of other macromolecules. The more dense phase is called the coacervate and the less dense phase is called the equilibrium solution (Bungenberg de Jong, 1949). The phase separation observed by Dill and Alsberg (1925), Haugaard and Johnson (1930) and Bungenberg de Jong and Klaar (1930a,b) fulfill the requirements of coacervation, and the phases with high protein concentrations of the gliadin preparations should therefore be called coacervates.

Clementz (1973) used salt as a mean to disperse WS-proteins from gluten. After treatment of gluten with 1 M NaCl, the WS-protein was dispersed in distilled water. We have found that 75% of the nitrogen containing material of gluten can be dispersed by this method (Carlson and Eliasson)¹. The effect is not osmotic in nature, since replacing salt with glucose did not work. It was shown by electrophoresis that the dispersed proteins consisted mainly of gliadin subunits, which were not covalently linked by disulfide bridges. The microscopic appearance of the dispersion obtained from this aqueous solution is identical to the 'separation drops' obtained in aqueous solution using gliadins extracted by organic solvents. The protein droplets were subjected to ultracentrifugation (3000 rpm, 1 h, 20°C) to yield a homogeneous yellowish pellet. This pellet was examined in polarized and normal light with microscope, and the optical anisotropy of the pellet is demonstrated in the micrographs of Fig. 17. The pellet is optically homogeneous and it probably represents the stable phase of the dispersed droplets. Low-angle X-ray analysis of the pellet did not show any diffraction spacing. Although there must be an order as evidenced by the birefringence, this indicates that the order is not regular enough in relation to the X-ray wavelength to give a diffraction. Thus there exists some kind of long range order in the pellet, the nature of which is still unknown. WS-proteins in aqueous solution thus separate reversibly with increasing concentration into a protein-rich and protein-poor phase. A dispersed protein-rich phase, a coacervate, is formed both from gliadin and total extracts of WS-proteins. Birefringent phases are observed for A-gliadin and for the sedimented coacervate of WS-proteins fractionated by salt.

¹ unpublished results

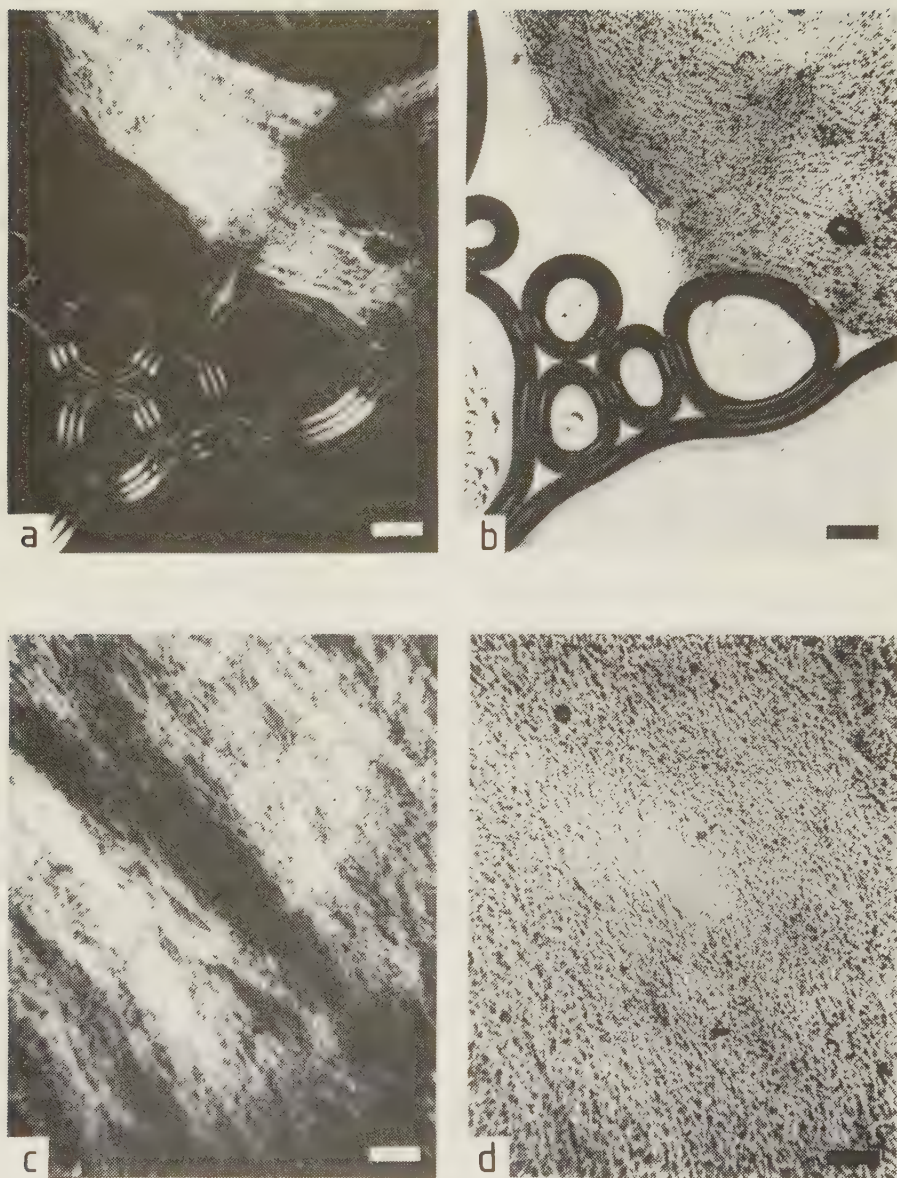


Fig. 17. Photomicrographs of pellet from sedimented droplets of wheat storage protein in water as observed in polarized light (a and c) and in normal light (b and d). Bar 233 μm .

3.3 Discussion regarding the conformation or structure of WS-proteins

Protein structures are often described in terms of models such as random coil, globular or rod-like. A linear macromolecule is said to be randomly coiled if the polymer exhibits little resistance to rotation about the bonds in the chain backbone and if there is little side-group interaction (Tanford, 1961). In a random coil solute-solvent interactions are dominant. A random coil does not adopt any unique three dimensional structure (high flexibility) and its conformation can be characterized by an average property, the average radius of gyration (Tanford, 1961). Solute-solute interaction of polymers in a random coil conformation must be virtually absent at low solute concentrations. Globular or rod-like proteins are often very compact and have a unique molecular shape. For these proteins intramolecular interactions dominate, and the solubility of these proteins depends primarily on solvent interactions on the protein surface. The packing efficiency in terms of packing density (actual volume/occupied volume) is in the order of 0.02-0.06 for randomly coiled polymers (Tanford, 1961), while it is around 0.70 for highly structured proteins (Richards, 1974).

The conformation of WS-proteins at low protein concentration and in strongly dissociating solutions is not likely to be a random coil, since these proteins tend to aggregate under such conditions as judged from sedimentation equilibrium experiments (Sexson et al., 1978). Optical rotary parameters for WS-proteins in 3 M urea indicate an α -helix content of between 10 and 20% (Wu et al., 1967). These indications of intra- and intermolecular interactions at low protein concentrations suggest that the WS-proteins examined are not in a random coil conformation in aqueous solvents. The disulfide bonds of these proteins also counteract the adoption of a random coil state.

4 COLLOIDAL PROPERTIES OF DOUGH AND GLUTEN

The colloidal properties of dough and gluten, considering these properties of their protein and lipid fractions, will be discussed here. ESP, flow properties and colloidal morphology of dough and gluten will be considered, and on this basis a colloidal model of dough will be proposed.

4.1 Dough and gluten as colloidal dispersions

An average dough, made from wheat flour and water (10 g flour and 5 g water) consists by volume of $\approx 60\%$ starch granules, $\approx 10\%$ air cells and $\approx 30\%$ of a mainly continuous hydrated matrix (VI). The starch granules and the air

cells are dispersed phases. The starch granules have as mentioned earlier a bimodal size distribution with size averages of around 10 and 25 μm , respectively (Meredith et al., 1978). The smaller starch granules are often spherical, whereas the larger ones frequently are lenticular in shape. The air cells have an average size of around 50 μm (VI). The total surface area between the continuous matrix and the dispersed phases is estimated to be 0.804 m^2 per g of dough ($\approx 43\%$ of water by weight). Of this is 0.8 m^2 on the surface area between starch and continuous matrix and 0.004 m^2 between air cells and continuous matrix, respectively (VI). The wheat/water system without dispersed starch granules is generally referred to as gluten. The continuous matrix consists of an aqueous protein/lipid matrix with an approximate chemical composition of 66% water, 29% proteins and 3.5% of lipids by weight. Other minor components, like pentosans, have not been considered here. The average thickness of the continuous matrix in the dough is calculated to be in the range of one μm , assuming that the matrix is evenly distributed over the surface of the dispersed phases (VI).

4.1.1 Wheat storage proteins

In view of the monolayer studies of DGDG and A-gliadin discussed in 3.1.3 it is reasonable to assume that no molecular interaction takes place between lipids and proteins, except when they carry a net charge of opposite signs. Since only 2-3% (w/w) of the polar lipids (Table 2) have a net charge at the usual pH of a dough (pH 5-6) the possible effect of such interactions can be disregarded as a first approximation. Thus WS-proteins and wheat lipids can be regarded as separate phases in dough and gluten, which may interact as is evident from the monolayer studies (3.1.3). According to the discussion in 3.1.2 and 3.2.3 the WS-proteins can be regarded as lipid-like amphiphiles. Because of this feature these proteins have strong tendency to aggregate. The continuous matrix of dough can therefore be described as a concentrated aqueous protein phase similar to a coacervate. Electrostatic interactions between glutenin and gliadin proteins at a pH between 5.5 and 6.5 are also expected, since these components have opposite net charges in this pH-region. The more detailed structure of the protein phase is not known. Only two structures seem to be possible, either lamellar or rod-like. A lamellar structure seems to be the most likely one based on the data presented in 3.1.2 and 3.2.3. This structure is also the most likely one from a functional point of view.

4.1.2 Wheat lipids

The possible physical states of the lipid material in dough and gluten is known from the phase diagram presented in 3.2.1. In flour the polar lipids are present in various membrane residues, and they are probably almost completely segregated from the non-polar lipids. When the flour is wetted in the initial stage of dough formation, lipids and water will make contact. Depending on the relative amounts of water, non-polar lipids and polar lipids different aqueous phases will form. All the possibilities can be deduced from the phase diagram presented. The dynamical process at the mixing stage is reflected in the relative amount of the phases at a given moment. If we regard the process as one where the polar lipids are exposed to increasing amounts of water, a reverse hexagonal phase is first formed. The mesophases formed will contain an increasing relative amount of non-polar lipids with increasing mechanical mixing of the dough. The lamellar mesophase formed is expected to accumulate, like any surfactant, at all surfaces, since thereby the surface energy can be lowered. There are two such possible surface locations at the air/water and the starch/water interfaces, and of these two the surface free energy at the air/water interface is dominating and accumulation there should thus be preferred. If also a L2-phase is formed, however, the amount of lamellar phase will decrease.

The existence of a lamellar lipid-water phase in dough is supported by the observation of a low-angle X-ray diffraction at 43-48 Å in dry and 52-56 Å in hydrated gluten, which was first reported by Hess (1954). Later Traub et al. (1957) found that this spacing disappeared as the lipids were removed from gluten. When samples were oriented parallel to the X-ray beam they observed a strong increase in the intensity of the reflections, and the increase was symmetric. Traub et al. (1957) suggested that the spacing was due to the presence of bimolecular lipid leaflets. Grosskreutz (1960) interpreted the low-angle spacing due to a lipid bimolecular leaflet and he also suggested a model of gluten (1961) based on such leaflets. This model has been cited frequently in literature. The effect of sample orientation suggests, however, that the spacing was caused by an aqueous-lipid lamellar mesophase.

We have also studied the spacing of gluten by low-angle X-ray diffraction (Carlson, Larsson and Mieziš)¹. We found that the spacing increased from 45 ± 1 Å to 55 ± 1 Å as the water content increased from $\approx 5\%$ to 67% (w/w). The 45 Å spacing was observed in dry gluten from all three wheat varieties studied, Amy, Starke, and Maris Huntsman. The diffraction line from wet gluten is weaker than that from the dry sample. This is probably caused by microbiological breakdown of gluten during the long exposure times used (1-4 days).

The maximum area of bilayers in dough can be estimated from the amount of non-starch lipids (Table 2), a bilayer thickness of ≈ 40 Å and a density of 1 g/cm^3 of the bilayer. One g of wheat flour dough ($\approx 43\%$ (w/w) of water) contains about 4 mg of polar lipids, and the area of the bilayer formed from these lipids is about 2 m^2 . As the total interfacial area in 1 g of dough is about 0.8 m^2 (VI), the thickness of the mesophase corresponds to about 2-3 bilayers. The diffraction spacing for a polar/non-polar lipid mixture of 4:1 is 49 Å and for only polar lipids 40 Å (I). The observed X-ray diffraction data from dry gluten then can be estimated to equal a phase at 7:1 ratio of polar to non-polar lipids. The aqueous swelling of this phase in gluten corresponds to a water content of about 25% (w/w).

4.1.3 Equilibrium spreading pressure of wheat constituents

The equilibrium spreading pressure (ESP) of flour and dry gluten at the air/water interface of different wheat preparations was studied. By measuring the ESP of complex systems such as flour and gluten, the components which stabilize the air/water interface in these systems can be identified, provided that the ESP-values of the individual surface active components of the systems are known. The ESP-values of these components were discussed above. Using the same method as described in (IV) the apparent ESP-values for flour, gluten and other wheat fractions were obtained (Table 9).

¹ unpublished results

TABLE 9

Equilibrium spreading pressures of flour, dry gluten and other derived wheat fractions (substrate 0.44 M NaCl, water $23 \pm 1^{\circ}\text{C}$).

Compound	ESP (mN/m)
Gluten (Starke II)	41
Flour (Starke II)	≈ 41
A-gliadin	25
Ethanol soluble WS-proteins (gliadins)	25
Wheat lipids (lamellar mesophase + L2)	42

It is evident from the results presented that the polar wheat lipids alone will determine the ESP of these samples.

4.2 Rheology of dough and gluten

The general aims of rheology for a system are to obtain:

1. A quantitative description of the mechanical properties.
2. Relations between observed rheological behaviour and the structure and composition.
3. Relations between observed rheological behaviour and functional properties.

Points 1 and 2 will be discussed for dough and gluten in this chapter and point 3 will be discussed in chapter 5. General rheological concepts can be found in Ferry (1961). Earlier rheological works on dough and gluten have been reviewed by Bloksma (1971) and Hibberd and Parker (1975).

4.2.1 Flow behaviour

Generally dough is considered as a non-linear viscoelastic material. This means that both stress, strain and time must be defined for a proper rheological description of dough. Although tensile measurements are possible to perform on both dough and gluten (Matsumoto, 1979; Funt Bar-David and Lerchenthal, 1975), simple shearing is more convenient to use. A simple relation exists between tensile and shear properties of rheological isotropic materials (Ferry, 1961). If this relation applies to dough and gluten is not known, however. In this study two types of loading patterns, both transient (stress relaxation) and dynamic in shear geometry, have been used. A new simple and

accurate stress relaxation instrument suitable for materials like dough and gluten was developed (VII). The flow behaviour of dough and gluten from the wheat variety Starke II has been determined in a stress relaxation experiment (VIII). The flow behaviour for strains between 0.1 and 1.0 is summarized below.

1. Both dough and gluten are non-linear materials as the relaxation modulus is strongly dependent on the strain. The relaxation function can be separated into functions of strain and time, for strains less than about 0.4 for dough and 1.0 for gluten.
2. The relaxation halflife for both dough and gluten is approximately 1.5 s. The relaxation modulus at 0.3 s, $G_{0.3}$, at a strain of 0.2 is $\approx 1800 \text{ N/m}^2$ for dough and $\approx 900 \text{ N/m}^2$ for gluten.
3. The relative stress in dough and gluten after 10^4 s is less than 3% and 0.5%, respectively, of the initial stress values for all deformations studied.
4. Two flow processes are apparent in the relaxation curves with relaxation times less and greater than approximately 10 s. The experimental relaxation results were analysed according to a cooperative flow theory (Bohlin, 1980). The observed relaxation curve is proposed to consist of two flow processes, both of which can be described as cooperative. The relaxation process at times less than 10 s is strongly cooperative, with a 4-fold coordination between the flow units. The second relaxation process at times larger than 10 s has a 2-fold cooperative coordination between the flow units. The cooperative strength between the flow units is about 4.6 kT and 1 kT in the first and second flow processes, respectively, as calculated from this theory (Bohlin, 1980). The 4-coordinated flow process transforms roughly 75% of the total stress into thermal energy both in dough and gluten.

Thus the rheological behaviour of dough and gluten at deformations less than about 0.4 and 1.0 can be described by the following parameters:

1. $G_{0.3}$, the relaxation modulus
2. $t_{1/2}$, the relaxation halflife
3. Z_1 , the coordination number of the first flow process
4. $(\sigma/\sigma_0)_{1-2}$, the relative stress level at which the two flow processes intersect as defined in (VII)
5. Z_2 , the coordination number of the second flow process

The flow behaviour of dough and gluten from different varieties of wheat was studied. For each variety the flow properties of dough and gluten followed the same trend as that observed for Starke (VII). This discussion will therefore be concerned mainly with gluten since this is a simpler system than dough. The experimental procedures were the same as in (VII) except when otherwise is stated.

The stress relaxation curves and the calculated rate-stress plots are shown in Figs. 18 and 19 for gluten samples obtained from the wheat varieties Amy, Starke II, Maris Huntsman, Clements, Pompe, Solid H, and a commercial wheat flour mixture. All curves in Figs. 18 and 19 appear to converge at long relaxation times to a stress level, which is practically zero. Thus, gluten must be described as a viscoelastic liquid. The characteristic rheological parameters for the gluten samples are collected in Table 10.

TABLE 10
Observed and calculated flow parameters for gluten samples
from different flours obtained from stress relaxation at
the strain 1.0 and temperature 23°C

Sample	$t_{1/2}$ s	Z_1 $\alpha \approx 0$	$(\sigma/\sigma_0)_{1-2}$ %	Z_2 $\alpha \approx 0.3$	$G_{0.3}$ N/m ²	Protein concentration g/ml
Amy	1.8±0.2	4.8±0.2	29±1	2	800±10	0.54
Solid H (1)	≈ 1.3	≈ 4.5	≈ 30	2	≈ 670	0.54
Pompe	≈ 0.8	≈ 4.2	≈ 26	2	≈ 670	0.54
Starke II	1.1±0.4	4.2±0.2	21±1	2	630±30	0.52
Clements	≈ 0.3	≈ 4	≈ 20	2	≈ 470	0.53
Maris Huntsman	0.3±0.1	3.6±0.2	19±1	2	500±10	0.52
Solid H (2)	≈ 0.3	≈ 3.3	≈ 15	2	≈ 460	0.51
Beagle	≈ 0.5	≈ 3.0	≈ 15	2	≈ 750	0.45
Commercial flour mixture	≈ 1.8	≈ 5.0	≈ 31	2	≈ 870	0.53

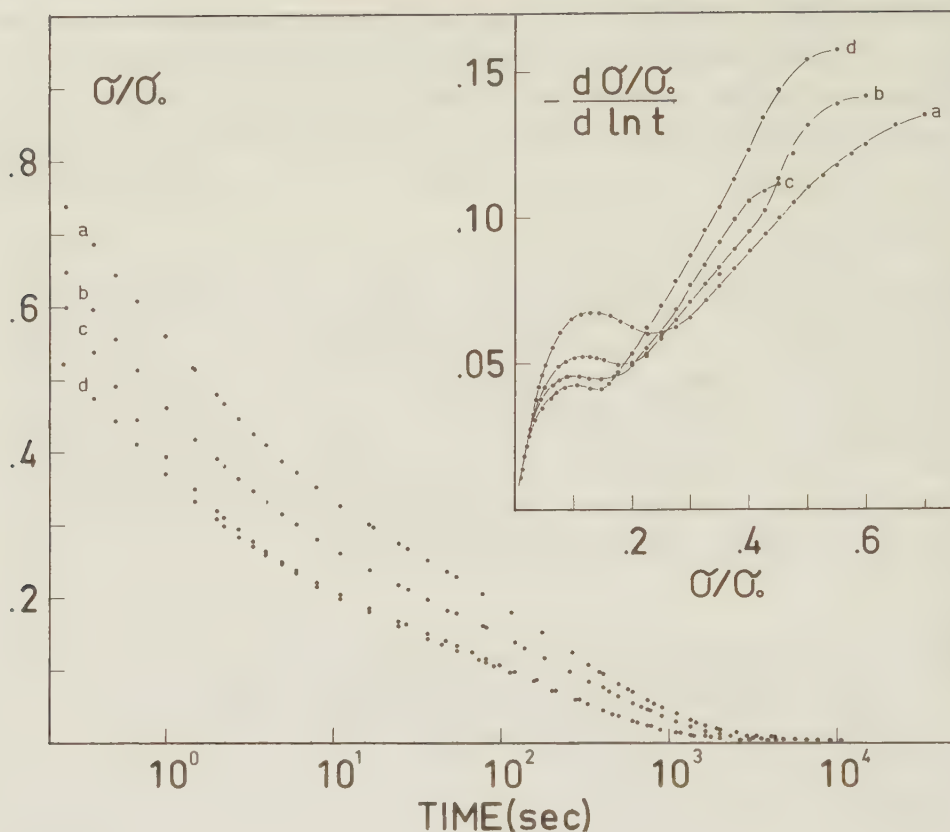


Fig. 18. Stress relaxation curves and calculated rate-stress plots for gluten samples at the shear strain 1.0. 1. Amy; 2. Starke II; 3. Clements; 4. Maris Huntsman.

The rate analysis of the relaxation experiments in Figs. 18 and 19 was performed as described in (VII) except that the second flow process in gluten was analysed using $Z_2 = 2$ and $\alpha \approx 0.3$.

The flow parameters in Table 10 vary between wheat varieties except for Z_2 . These four flow parameters vary for the different varieties in such a way that these parameters correlate positively with each other (Fig. 20). The observed differences between wheat gluten samples in flow parameters are expected to be due to qualitative differences, since the protein concentrations of the gluten samples do not differ much from each other. It is believed that the linear relations shown in Fig. 20 are correct even though the true values of G and $t_{1/2}$ differ from those observed. The fact that σ_0 as experimentally observed is 'contaminated' with relaxation, i.e.

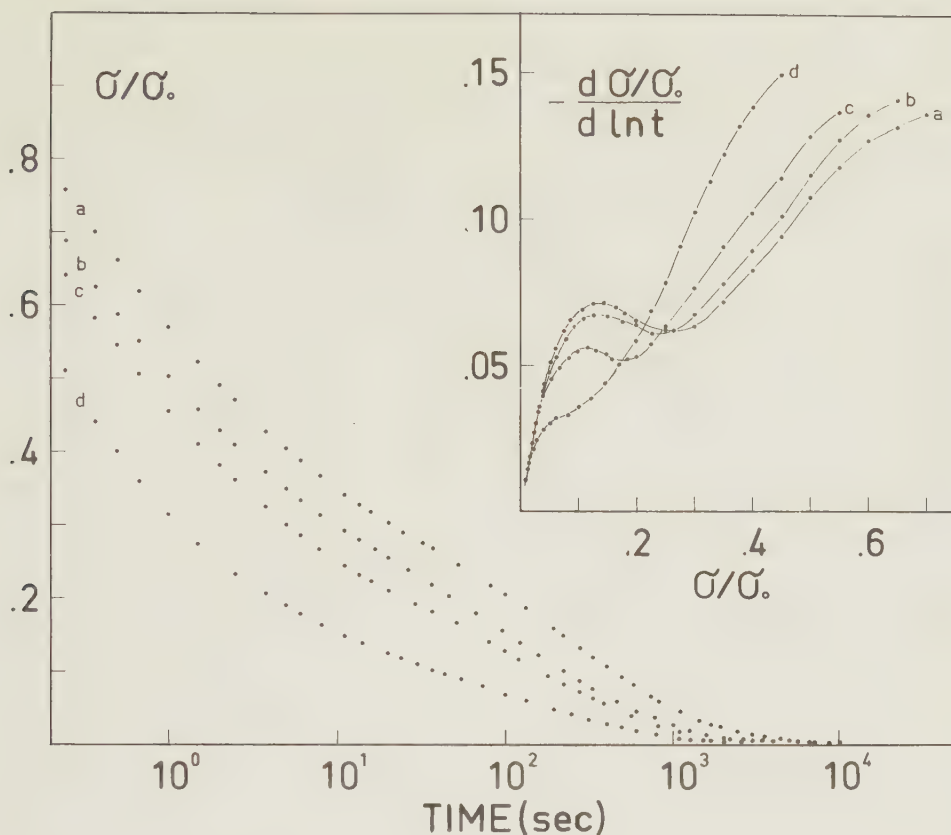


Fig. 19. Stress relaxation curves and calculated rate-stress plots for gluten samples at the shear strain 1.0. 1. Commerical wheat flour mixture; 2. Solid H (1); 3. Pompe; 4. Solid H (2).

stress is relaxed during the deformation of the sample, will give lower apparent G and greater $t_{1/2}$ values than those which would be observed if σ_0 was independent of time.

The flow parameters of two different samples of gluten from Solid H were also studied; Solid H (1) was grown in Svalöv and Solid H (2) in Hammenhög. It is evident from the flow parameters of these samples (Table 10) that the growing and harvesting conditions may strongly influence the rheological behaviour of gluten and dough. For a comparison the flow behaviour of gluten and dough from triticale (Beagle) and from a rye (Othello) dough ($\gamma = 0.2$) is shown in Fig. 21. Gluten from Beagle shows a similar flow behaviour to that of gluten from Clements and Maris Huntsman. The rye dough, which was composed of 10 g flour (Othello) mixed for 5 min, at 60 rpm with 7.4 ml water, gave a very high value of $G_{0.3}$ ($\approx 4000 \text{ N/m}^2$, $\gamma = 0.2$) compared to that of the

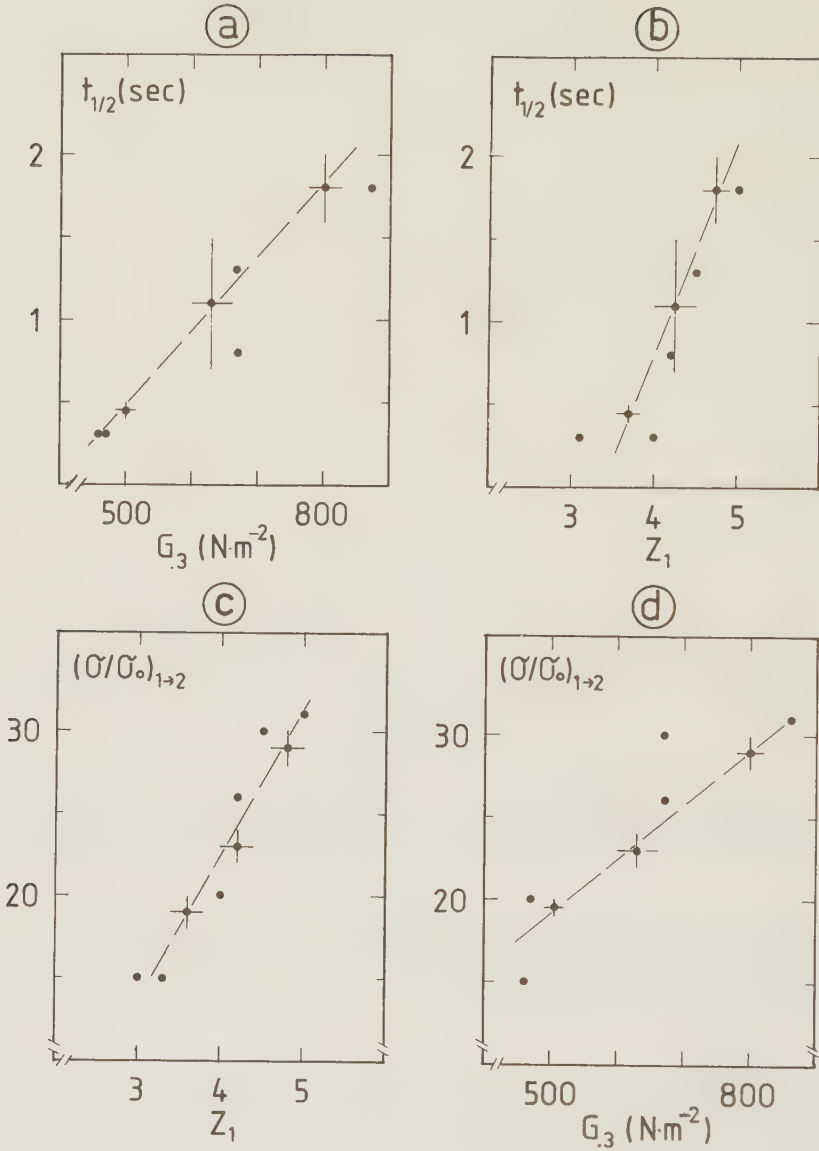


Fig. 20. Four flow parameters; the relaxation modulus ($G_{0.3}$), the relaxation half-life ($t_{1/2}$), the coordination number of the first relaxation process (Z_1) and the relative stress level at which the two flow process meet $(\sigma/\sigma_0)_{1 \rightarrow 2}$, are shown as function of each other for the different wheat gluten samples studied (Table 10).

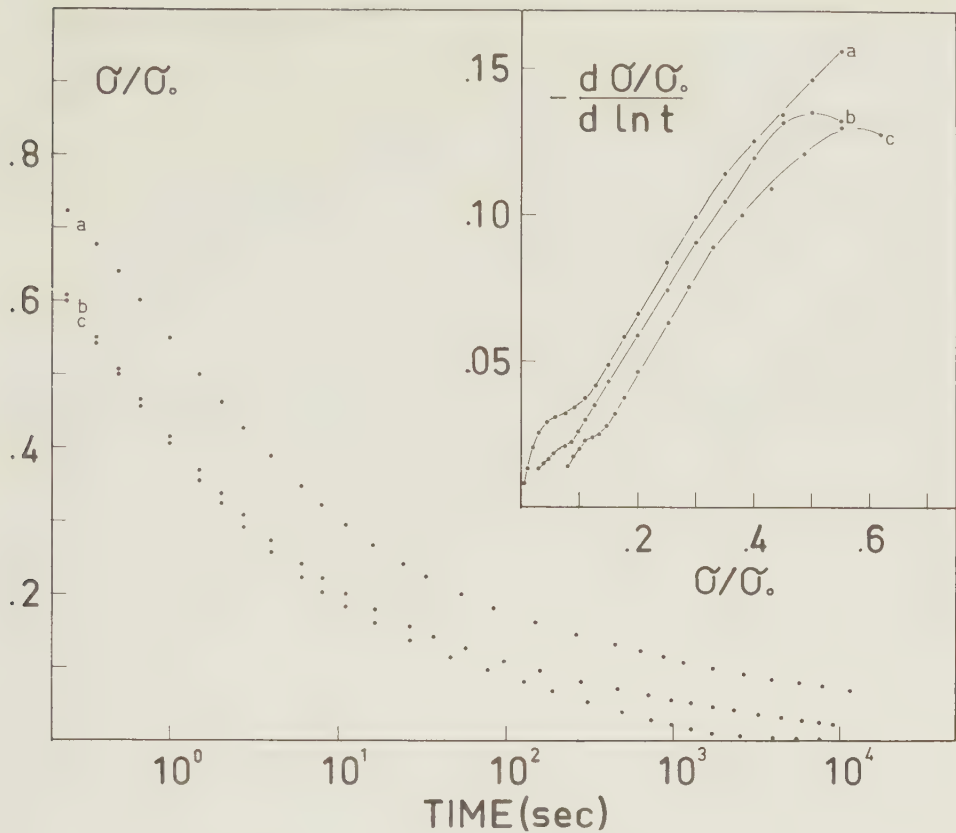


Fig. 21. Stress relaxation curves and calculated rate-stress plots of dough samples from Beagle (triticale) and Othello (rye) and from gluten from Beagle. 1. Gluten from Beagle, $\gamma = 1.0$; 2. Dough from Beagle, $\gamma = 1.0$; 3. Dough from Othello, $\gamma = 0.2$.

wheat doughs at the same deformation (VII). The rate-stress plot for the rye dough (Fig. 21) is different from those observed for wheat doughs at corresponding deformations. The rate-stress curve for the rye dough is displaced along the stress axis in such a way that the linear parts intersect the stress axis at positive stress value. A similar displacement was observed for wheat dough at a deformation of 1.0 (VII). This anomalous behaviour may reflect that a mechanical rupture had occurred in the dough.

4.2.2 Flow behaviour and structure

Possible relations between flow behaviour and relevant 'structures' in gluten and dough are discussed below, starting with a review of concepts and relations between flow properties and 'structures' for non-crosslinked polymer melts and solutions.

The entanglement concept in polymer rheology. The rheological behaviour of polymers is often presented in the form of a relaxation spectrum (Ferry, 1961). The distribution function of relaxation times $H(t)$ is approximately equal to $-\frac{d\sigma/\sigma_0}{d\ln t} \cdot (\sigma_0/\gamma)$. A schematic relaxation spectrum of polymers (non-crosslinked) are shown in Fig. 22. The different parts of a relaxation spectrum are called the glassy, transition plateau and terminal zones as indicated in Fig. 22. The relaxation in the transition zone is very similar for most polymers and is independent of the molecular weight of the polymer (Ferry, 1961). The transition zone is fairly well described by the Rouse theory (Rouse, 1953). This theory, which is based on a bead-spring model predicts a slope of $-1/2$ of the transition zone in the relaxation spectrum, as often observed for random coil polymers. The transition zone is believed to depend on short-range cooperative motions in small parts of a flexible random coil polymer. The plateau zone observed for a non-crosslinked random coil polymer is due to entanglement coupling. Graessley (1974) regards chain entanglements as intermolecular interac-

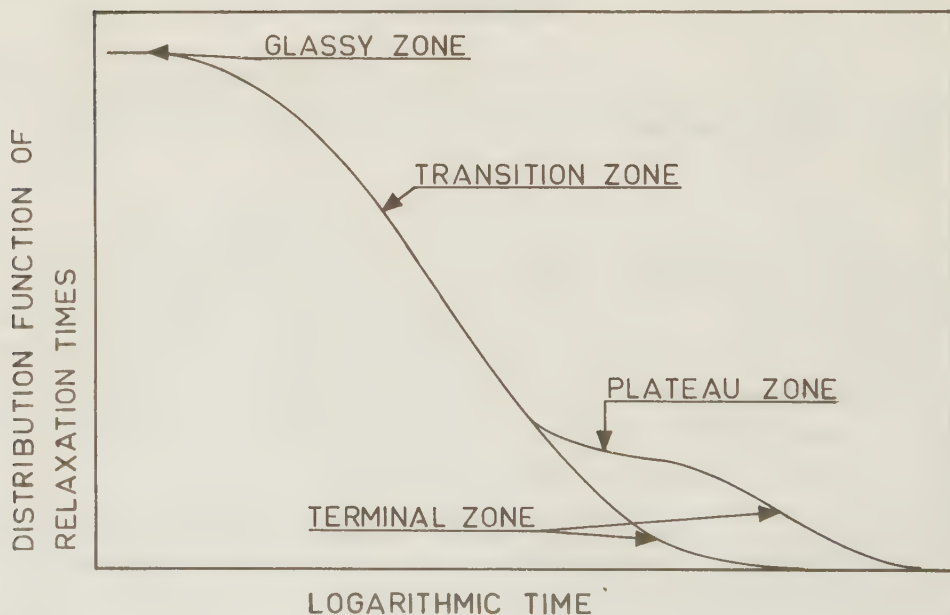


Fig. 22. Schematic stress relaxation spectrum of a non-crosslinked polymer (melt or concentrated solution).

tions which produce a transient polymer network. This network affects mainly the large-scale motion of the polymer chains. The mechanical strength of the transient entanglement network can be expressed as the apparent average molecular weight between entanglement coupling junctions, M_e . M_e for a polymer solution can be described by the following equation (Grassley, 1974),

$$M_e = \frac{c \cdot R \cdot T}{G_{\text{plateau}}} \quad (2)$$

where c is the polymer concentration, R the gas constant, T the temperature and G_{plateau} the observed modulus at the plateau. The characteristic relaxation time of the terminal zone (τ_m) is proportional to $\eta_0/c^2 \cdot T$ (Graessley, 1974) where η_0 is the zero-shear viscosity. The Rouse theory predicts τ_m from the relation

$$\tau_m = \frac{6}{\pi^2} \cdot \frac{\eta_0 M}{c \cdot R \cdot T} \quad (3)$$

which gives relaxation times in the same order as those observed. Typical relaxation times (τ_m) are in the range 10^{-4} - 10 s for homologous synthetic polymers and 10^{-6} - 1 s for protein solutions (Menjiuar and Rha, 1980).

A recently presented molecular theory by Doi and Edwards (1978a, 1978b, 1978c, 1978d) based on a primitive chain model explains the rheological behaviour of polymer melts and concentrated polymer solutions in a new way. The effect of a high polymer concentration on the rheological behaviour is that the mobility of a polymer chain is highly anisotropic due to the surrounding polymers. This is described geometrically as if the polymer chain is forced to move in a hypothetical tube. The tube represents the collective topological effect of the surrounding chains and its motion is therefore much slower than the individual chain. Doi (1980) has generalized the Doi-Edward theory further to give three kinds of relaxation processes, each of which has a characteristic time;

1. τ_1 is the relaxation time which corresponds to the relaxation within a chain segment between entanglement points. Here $\sigma(t)$ is proportional to $t^{-1/2}$.
2. τ_2 , which is proportional to $\tau_1 M^2$, is the longest relaxation time that the polymer chain would have in the absence of entanglements. This process is present only in the non-linear rheological region at large strains.

3. τ_3 , which is proportional to $\tau_1 M^3$, is the characteristic relaxation time of the entanglement points.

The relaxation spectra for gluten from Amy, Starke II, the wheat flour mixture and Maris Huntsman are shown in Fig. 23. The shape of these relaxation spectra is similar to those observed for concentrated solutions of flexible random coil polymers of high molecular weight in the sense that a plateau zone is observed, which is followed by a terminal zone. There are important differences between these polymer systems and dough and gluten, which indicate that the molecular entanglement model is unsuitable for gluten and dough.

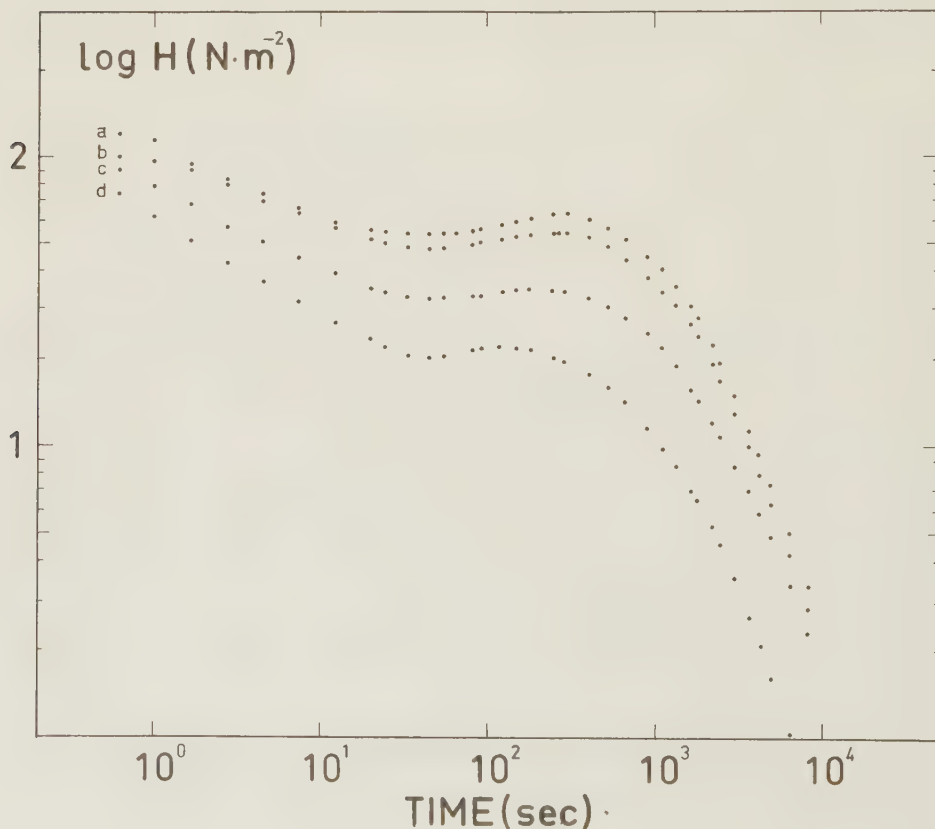


Fig. 23. Calculated stress relaxation spectrum of gluten samples at the strain of 1.0. 1. Commercial wheat flour; 2. Amy; 3. Starke II, 4. Maris Huntsman

- The entanglement model is based on polymers in a flexible random coil, a very unlikely conformation for the WS-proteins in an aqueous system.
- The relaxation time of the terminal zone for gluten is in the range of $10^2 - 10^4$ s. This range is $10 - 10^3$ times larger than the relaxation time usually observed for polymer solutions (Menjiuar and Rha, 1980).
- The frequency dependence of the modulus for dough and gluten is of the form $\omega^{-1/4}$ (VIII) at times corresponding to the transition region. This relation usually observed in the transition region of polymers is $\omega^{-1/2}$, which is also predicted by the Rouse theory (Rouse, 1953). Thus the relaxation in this zone for gluten and dough is not due to movements in the chain segments between the coupling points in the transient network.
- Using relation (2) for gluten (Starke II, $\gamma = 1.0$), with $c = 0.52$ mg/ml and the modulus of the plateau value from Fig. 23 we find that M_e is about $2 \cdot 10^7$. M_e must be smaller than the molecular weight M of the individual polypeptides and M_e is typically less than 20000 for polymers with molecular weights up to 150000 (Graessley, 1974). The molecular weights of the protein 'aggregates' in gluten which are responsible for the observed plateau must therefore be larger than $2 \cdot 10^7$. If we assume that the average molecular weight of the WS-protein is $5 \cdot 10^4$ we can estimate the minimum volume to be $64000 \text{ \AA}^3$ (Richards, 1977). The minimum size of a WS-protein aggregate, with an aggregate weight of $2 \cdot 10^8$, is approximately $0.1 \text{ \mu m}$, i.e. the aggregate is of colloidal size. Particles of this size is beyond the scope of the present molecular theories on transient networks of concentrated polymer systems.

The entanglement concept can be used not only for random coils but also, at least qualitatively, for other polymer conformations at high protein concentrations, since it is to be expected that polymer aggregates of colloidal size also will show anisotropic mobility under external stress. The observation (Helders et al., 1956), that the relaxation spectrum of aqueous sodium deoxyribonucleate exhibits a broad plateau, indicates that transient networks may also be found in concentrated solutions of polymer molecules, which are not in a random coil state.

Flow behaviour and colloidal nature. Possible relations between the colloidal nature and the observed flow behaviour of gluten will now be discussed. The continuous matrix is likely to dominate the flow behaviour at long relaxation times if we assume that this time correlates with the size of the flow units. Thus, the 2-coordinated flow process should have its origin in the flow be-

haviour of the relaxing units in the continuous matrix. The 4-coordinated relaxation process at short times is proposed to be due to the presence of inhomogeneities of colloidal size in the continuous matrix.

The continuous matrix. The 2-coordinated flow process is believed to depend mainly on flow in the continuous matrix. The flow units are 2-coordinated and the only reasonable 'rheological structure' with this coordination number is the lamellar structure. The concept 'rheological structure' indicates a dynamic structure, which dominates during the flow process, in contrast to a static structure. The rheological and static structures may be identical, as has been shown for some simple well-defined liquid-crystalline phases of lamellar and hexagonal type (Bohlin, 1979). The lamellar rheological structure of the continuous matrix of gluten and dough may correspond to a static structure of lamellar liquid-crystalline type, as judged from the optical birefringence of the concentrated aqueous phase of the WS-proteins.

The apparent differences observed in the rheological parameters and their positive linear correlation with each other (Table 10) can be explained if it is assumed that these parameters indirectly express some properties of the 2-coordinated continuous structure. These properties could be the size and/or the relative volume occupied by this 2-coordinated structure in the continuous matrix. The maximum in the relaxation spectrum of the 2-coordinated flow process and the time of this maximum are linearly dependent on each other (Fig. 23) as well as on the other flow parameters (Table 10) as is shown in Fig. 24. The flow behaviour of gluten is thus believed to be described by the size and relative volume of the 2-coordinated aqueous protein phase in the continuous matrix, as expressed by the values of the flow parameters used.

The discontinuous phases. The flow units in the first relaxation process at short times should be expected to be considerably smaller than those of the continuous phase. The principal discontinuous phase of gluten is the air cells. The 4-coordinated cooperative flow process can be interpreted to be due to local flow in the continuous matrix under the influence of the dispersed air cells. The interpretation of the coordination number of this process according to Bohlin (1980) is that each relaxing flow unit on the average cooperates with four neighbouring units in the flow process. The relaxing unit may consist of deformed air cells together with surrounding continuous matrix. The first relaxation process 'consumes' about 75% of the

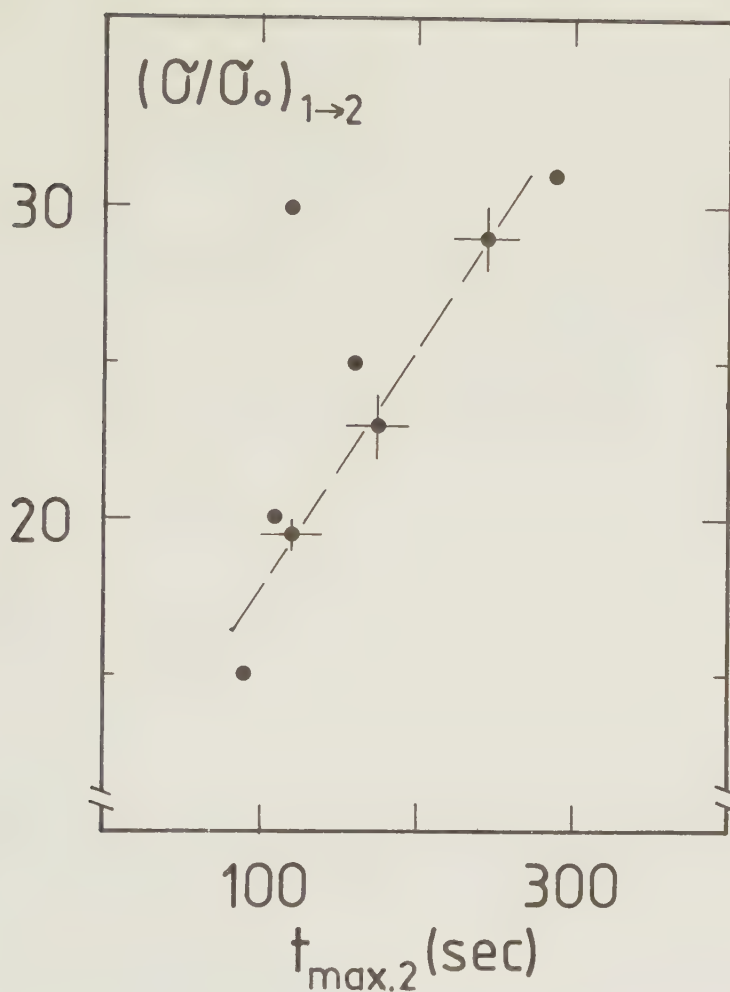


Fig. 24. $(\sigma/\sigma_0)_{1 \rightarrow 2}$, the relative stresses where the two flow processes meet are shown as a function of the time at the maximum in the relaxation spectrum of the second relaxation process ($t_{\max.2}$) for the different wheat gluten samples studied (Table 10).

total stress during the relaxation process. This means that the discontinuous phase dominates the rheological behaviour when stress is compared. The continuous matrix, however, dominates the total relaxation when time is considered.

The influence of the air cells on the elasticity of dough has been discussed in (V) where the time aspect, however, was not considered. In light of the stress relaxation results (VII), it is clear that the time aspect is very important. Therefore any discussion of pure elasticity of dough and gluten is incorrect in this time regime. The modulus used in (V) corresponds to a time of about 5 s, and this will therefore correspond to a relaxation modulus at short times. The basic assumptions in (V) were that stress is equal throughout the dough, and that the local modulus of the dispersed phase is much smaller than that of the continuous matrix. Lindenmeyer (1978) has recently shown that the assumption of constant stress is a relevant approximation when the dispersed phase is of spherical shape. He gives the following relation (Tsai-Halpin relation) between the geometric and rheologic parameters of the total system and those of the different phases:

$$G = G_A \cdot \frac{1 + \xi \cdot \chi \cdot V_B}{1 - \chi \cdot V_B} \quad (4)$$

where G is the modulus, V_B is the volume fraction of the continuous phase

$$\chi = \frac{G_A - G_B}{G_A + \xi \cdot G_B} \quad (5)$$

ξ is the contiguity parameter, A dispersed phase and B continuous phase, respectively. The contiguity parameter (ξ) is a measure of the morphology of the material and it allows an extrapolation between the two extreme cases of constant stress and strain. For continuous fibre materials ξ is defined as the length diameter ratio of the fibres, and consequently ξ becomes very large. The infinite large value of ξ represents a condition of constant strain. At spherical geometry of the dispersed phase ξ equals one. This value approximately equals the value of ξ for constant stress. An example of equation (4) is shown in Fig. 25. For $G_B/G_A = 100$ and $V_A = 0.1$ we find $G/G_A = 9.2$. This value of G/G_A corresponds to a situation when approximately 90% of the total strain is produced in the discontinuous phase.

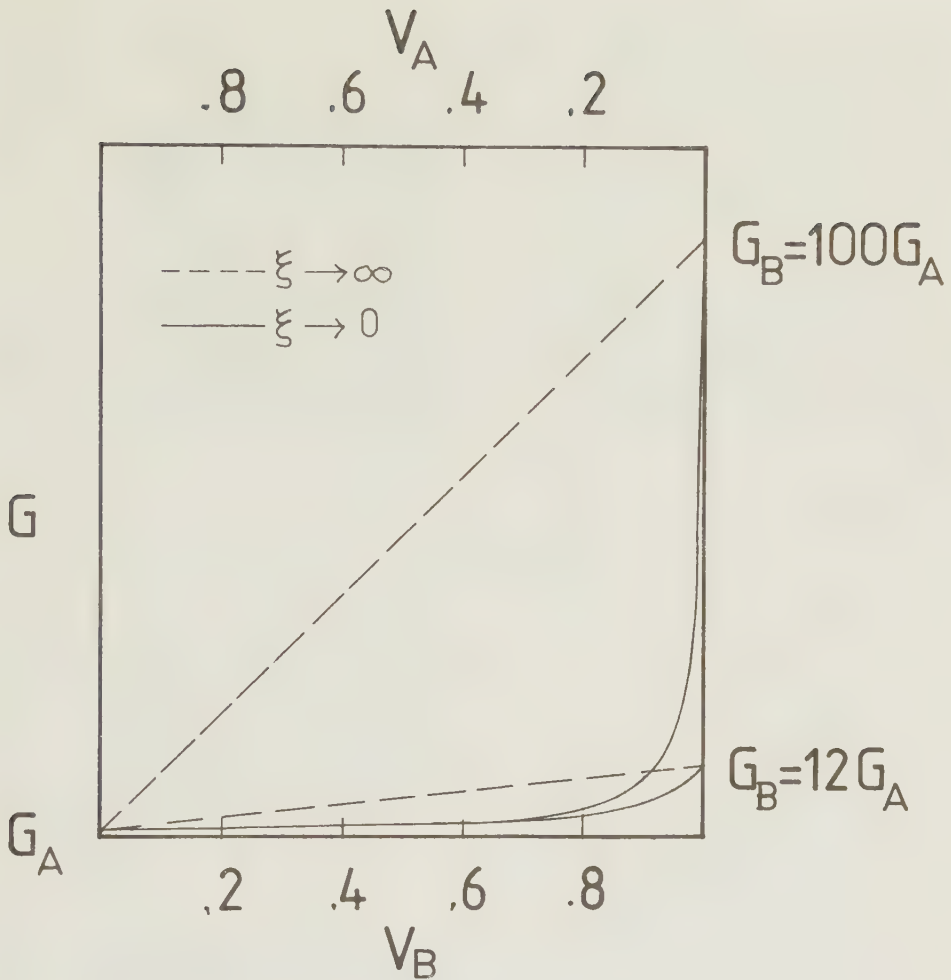


Fig. 25. The use of the Tsai-Halpin relation (equation (4)) for interpolating between the two extreme models, homogeneous strain ($\xi \rightarrow \infty$) and homogeneous stress ($\xi \rightarrow 0$) for an isotropic material. G is the modulus, V_A and V_B are the volume fractions of the continuous phase and the dispersed phase, respectively. Two examples are shown with different ratios between the two modulus G_B and G_A .

The initial applied strain in stress relaxation is constant during the experiment. The morphology of gluten and dough is such that the total stress is constant and evenly distributed in the heterogeneous material. Thus the strain will be locally distributed in relation to the different values of the modulus within the dough. The relative stress 'consumed' by the first relaxation process (approximately 75%) represents the discontinuous phase in

terms of its volume fraction, strain and modulus.

The influence of starch on the flow behaviour can be illustrated by a comparison between dough and gluten (VII). The presence of starch primarily influences $G_{0.3}$, which is increased. There is also a change in the second flow process which becomes more strongly cooperative when starch granules are present. This might be due to the orientation of the continuous matrix since this will be sheared between the starch granules.

4.3 A qualitative colloidal model of wheat flour dough

Based on the presented properties of wheat lipids and wheat storage proteins at the air/aqueous interface and in the aqueous bulk, a qualitative colloidal model can be proposed. The general appearance of a section of wheat flour dough observed in a light transmission microscope is shown in Fig. 26a. The well known dispersed phases of starch granules and air cells in the continuous matrix are observed at this magnification. At a higher magnification lipids and aqueous lipid aggregates are observed together with small air cells and starch granules. Small air cells have been observed in transmission electron microscopy by Betchel et al. (1978). Both non-polar lipids and aqueous lamellar mesomorphic particles are dispersed in the continuous matrix. The interfaces between the dispersed phases and the continuous matrix are believed to be covered with a lamellar mesophase as is indicated in the picture. The existence of polar lipids on the surface of fractionated starch granules has been demonstrated (Eliasson et al., 1981). At the highest magnification shown the general structure of the aqueous lipid mesophase is evident. The aqueous protein structure of the continuous matrix is lamellar with polar regions consisting of water and polar parts of the protein molecules segregated by non-polar regions of the proteins.

5. COLLOIDAL NATURE AND BAKING PERFORMANCE OF WHEAT FLOUR DOUGH

5.1 Introduction

The most important utilization of the technical properties of the wheat flour dough is the production of bread, mainly of the white fermented type. The ability of wheat flour to produce loaves of high specific volume is a general accepted criterion of the baking quality of the flour. Baking quality as assessed by a baking test is only relative, since the actual result depends on the outline of the test used. The baking performance of a wheat flour depends, besides the baking test conditions, on genetic species variations and the environmental conditions during growth, on storage conditions after har-

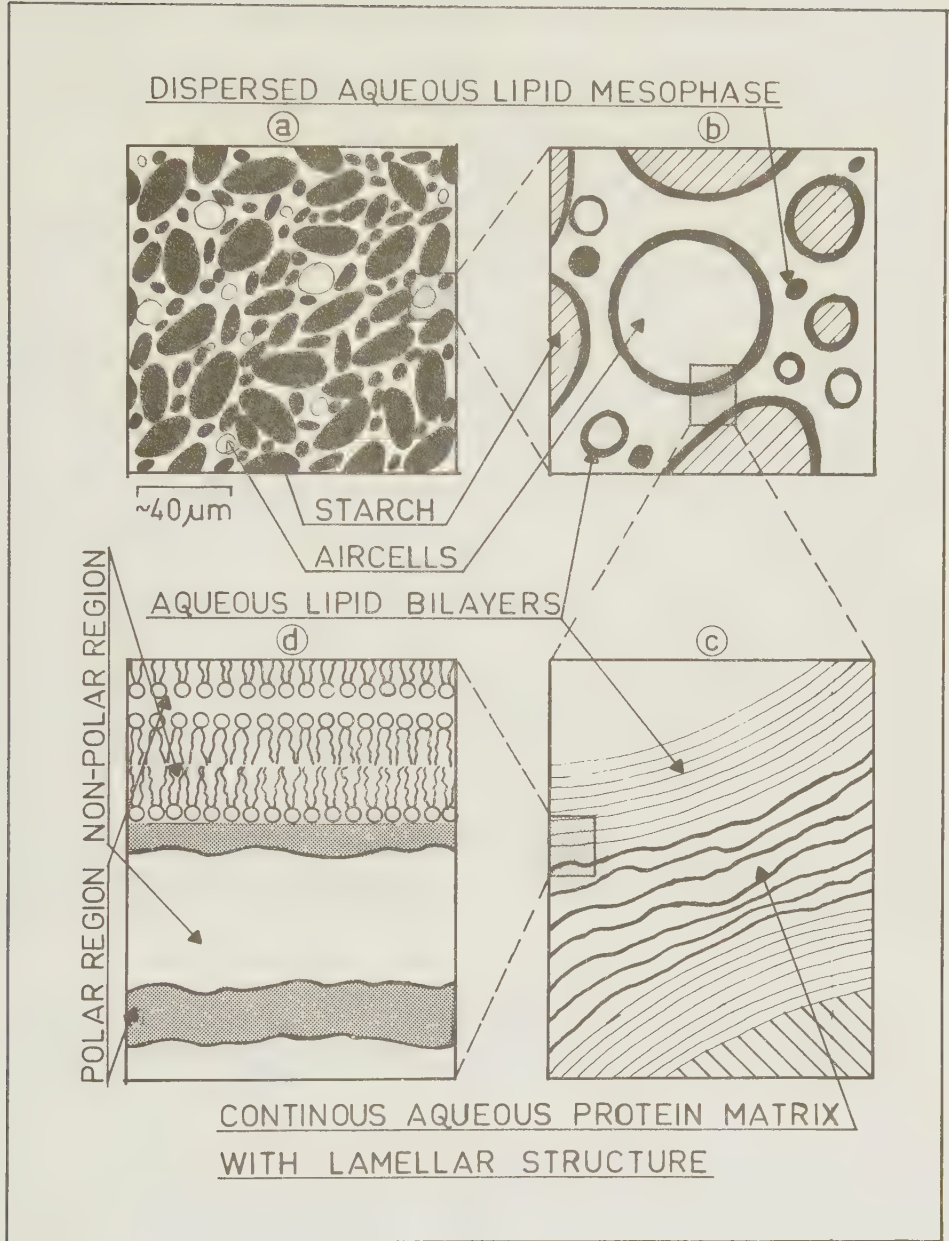


Fig. 26. Schematic illustration of a qualitative model of wheat flour dough at four different levels of magnification.

vesting, and finally on the manner in which the kernals are milled. A simple bread making process can be divided into three main parts:

1. The mixing of the proper ingredients into a dough. It is believed that the necessary flow and gas-holding properties of the dough are created to a large extent already at this stage.
2. The fermentation process, where the dough is internally deformed by the gas produced by the yeast. The flow and gas-holding properties are utilized in this process. Intermittent process steps are normally used for shaping the dough before the final fermentation period.
3. The final baking process, where the fermented dough is subjected to heat, which at first leads to a thermal volume expansion of the dough, and finally the rigid bread structure is formed, as the starch granules partly gelatinize.

5.2 Dough and loaf volume

The amount of gas entrapped into the dough during mixing is about 10% (v/v) (V), which means that the dough at this stage of processing is best described as a gas cell dispersion or 'Kugelschaum' (Bikerman, 1973). This gas dispersion is transformed into a foam with about 90% (v/v) of gas during the fermentation. The gas volume increases still further as a consequence of the thermal expansion during the early part of the oven stage. In the last part of the oven stage, the dough foam is converted to a sponge, in which the majority of the closed foam cells has become opened as a consequence of the rupture of the foam lamellae.

5.2.1 Effects of foam stabilizer and destabilizer

The characteristic geometrical change of the dough during the fermentation and baking stages is the large increase in the gas/liquid interface area. This increase in area is accompanied by a decrease in the thickness of the continuous matrix. The volume of the expanding dough will to a large extent depend on the mechanical stability of the continuous matrix during the expansion which is believed to depend both on surface and bulk properties of the matrix. By baking doughs to which foam destabilizers or stabilizers have been added we may get information on this question of the stability of the continuous matrix.

Materials and methods. The lipid materials and their physical state in which they are used are listed in Table 11.

TABLE 11
Specifications of the additional materials used
in the baking test

Material	Physical state
Epicuron a very pure fractionated soybean lecithin (about 99% lecithin) from Lucas Meyer, Hamburg, BRD	Added to the flour in the form of a lamellar liquid-crystalline disper- sion in water (liposomes) before the other dough ingredients were added
Silicone oil (polydimethylsiloxane) MS 200, viscosity of 1000 cp, from Kebo-Grave, Sweden	Added in the form of an aqueous emulsion to the dough liquore
Trilaurin from Fluka AG, Switzerland	Added to the flour
Dimodan P monoglyceride of hardened lard con- taining 90% (w/w) of 1-monoglyceride and 10% (w/w) 2-monoglyceride from Grindstedvaerket A/S, Brabrand, Denmark. Melting point $\approx 70^{\circ}\text{C}$	Added alternatively to the dough li- quid or to the flour in the form of an aqueous gel
Panodan AB 90 diacetylated tartaric acid ester of monoglycerides from Grindstedvaerket A/S, Brabrand, Denmark. Melting point $\approx 20^{\circ}\text{C}$	Added to the flour in the form of a lamellar liquid crystalline dispersion in water (liposomes) before the other dough ingredients were added

The baking method used in our laboratory is based on that used by the Swedish Seed Association in Svalöv (Olared, 1979). Unless otherwise stated the for-
mulation for test loaves was as follows:

Flour (dry weight)	about 200 g
Yeast	8 g
Ascorbic acid	10 mg
Sodium chloride	1.9 g
Sugar	5.0 g

For flours with falling numbers higher than 250 0.2% of malt flour was added on dry weight. All the ingredients of the dough were brought to 23°C before the start of the baking test. Most of the flour was transferred to the mixing bowl (Farinograph bowl, 300 g) and mixed for one minute. The dough liquid, in-

cluding the yeast, the sodium chloride, the sugar and the ascorbic acid and made up to 125 ml with distilled water, was added to the flour. The consistency of the dough was adjusted to 400 BU by adding the necessary quantity of the remaining flour. The mixing was continued until the peak value was reached on the farinogram. The speed of the mixer was 62 rpm.

The dough was fermented in plastic bowls at 32-35°C (98% r.h.) for 55 to 60 minutes. After punching by hand the dough was divided into three pieces of each 100 g, which were molded 25 revolutions on the molder and placed in the greased pans. The dough pieces were fermented 70 minutes at 37°C (98% r.h.). The breads were baked for 20 minutes at 240°C. The bread volumes were determined on a Fourneau measuring device made of plexiglass with sago seed. The standard deviation ($n = 5$) of the loaf volumes in this baking test was found to be less than about 15 ml/100 g flour. The amount of flour (dry weight) used in the baking test was: Amy 192 g, Starke 203 g, Maris Huntsman 193 g, and wheat flour mixture 186 g. Some chemical and physical characteristics of these flours are shown in Table 12.

TABLE 12
Chemical and physical characteristics of four wheat
flours used for baking

Wheat flour	Extraction rate %	Protein content %	Falling number s
Amy (1975)	66	13.5	383
Starke II (1975)	68	10.0	367
Maris Huntsman (1975)	67	11.5	182
Commerical wheat flour mixture	-	12.6	250

Results and discussion. Polydimethylsiloxane, which is a well known foam inhibitor in many different foams (Kitchner and Cooper, 1959) was chosen as a substance for testing the foam behaviour of dough. Polydimethylsiloxane has a very low solubility in water and a surface tension of 20 mN/m. The surface pressure and area at collapse of polydimethylsiloxane is approximately 8 mN/m and 0.63 m²/mg, respectively, at the air/water interface (Noll et al., 1971). Concentrations of the order 1 - 60 ppm prevent foaming (Kitchner and Cooper, 1959). The exact mechanism by which polydimethylsiloxane inhibits foam is unknown. The effect on bread volume of added polydimethylsiloxane is

shown in Fig. 27 for doughs made of the commercial wheat flour mixture. No so called oven rise can be observed with polydimethylsiloxane, which means that the total increase in volume is produced during the fermentation. The observed effect of polydimethylsiloxane on dough volume is that to be expected if the fermented dough is regarded as a foam. It should be mentioned that polydimethylsiloxane has no observable effect on the gas producing capacity of the yeast cells in the concentrations used here. At concentrations of polydimethylsiloxane above approximately 100 ppm an almost constant loaf volume is observed which indicates that the interfaces of the foam lamellae of the dough contain a monolayer (of about 4 m^2) of polydimethylsiloxane.

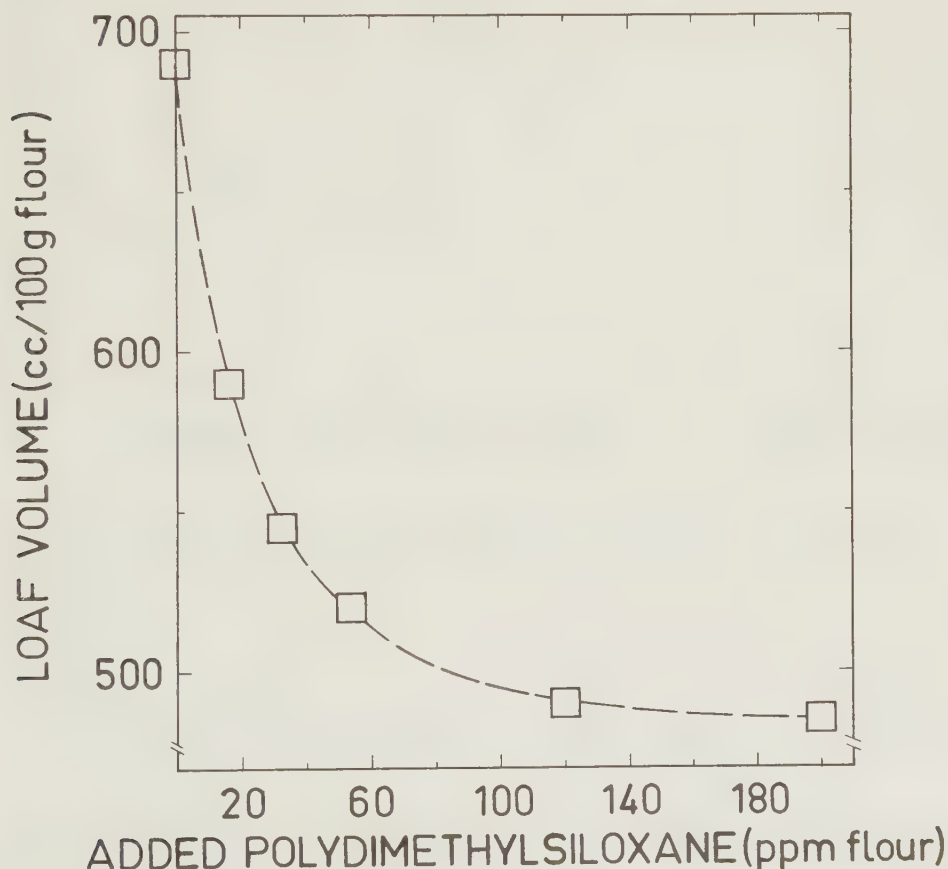


Fig. 27. Loaf volume as a function of added polydimethylsiloxane for the commercial wheat flour mixture.

PC in the lamellar liquid-crystalline phase was chosen as a foam stabilizer as this phase according to the earlier discussion is naturally occurring in dough. The lamellar mesophase has been proposed by Friberg (1978) to be a general structure, which stabilizes foams. The positive effect of the liposomedispersion of Epicuron on the loaf volume is drastic as is evident from Fig. 28. The maximal effect of the liposomedispersion is found at approximately 7%, calculated on the amount of dry flour. This amount of PC equals approximately $4 \cdot 10^5 \text{ m}^2$ of PC bilayers, an area which is approximately 10^5 times larger than that of the collapsed polydimethylsiloxane monolayer. The stabilizing effect of this mesophase on the foam structure of the dough must therefore involve other interfaces than the air/liquid interface of the foam. The solubility of dipalmitoylphosphatidyl choline is approximately 10^{-10} M (Smith and Tanford, 1972) and this value is believed to be representative for PC molecules with long hydrocarbon chains. The observed effect of Epicuron on loaf volume is thus not likely to be due to monomers of PC. The observed effect of PC must be due to the physical properties of the lamellar liquid-crystalline phase, since the same drastic effect also is observed with Panodan AB 90 (diacetylated tartaric acid esters of monoglycerides) in the lamellar liquid-crystalline state (Figs. 28 and 29). Dimodan P (distilled monoglyceride) was used in the form of its aqueous gel phase; the gel phase has the same lamellar organization as the lamellar liquid-crystalline phase except that the hydrocarbon chains are crystalline. The effect of the gel phase is much smaller when compared with that of the lamellar mesophase (Fig. 28). The gel phase gives a maximal increase in loaf volume which is equal to that observed when a fat (hardened soybean oil 4041, melting point $\approx 41^\circ\text{C}$, Karlshamns Oil Factory, Karlshamn, Sweden) is used at the 4% level. The observed increase in loaf volume of the addition of the gel phase and the fat is similar in size to the well known effects of shortening (Bell et al., 1977). The addition of trilaurin crystals to the dough has no significant effect on the observed loaf volume.

The observed effect on the dough volume of the lecithin mesophase is only apparent at the oven stage, which is in agreement with the observations reported by Baker and Mize (1939) and MacRitchie and Gras (1973) on the effects of added fat and wheat lipids, respectively. The reason for this lack of volume improvement with the addition of lipids during the fermentation stage is not understood at present. One possible explanation can be found if we look at the kinetics of the expansion of the dough volume in the fermentation and baking process. The rate of volume expansion in the oven stage is 5 to

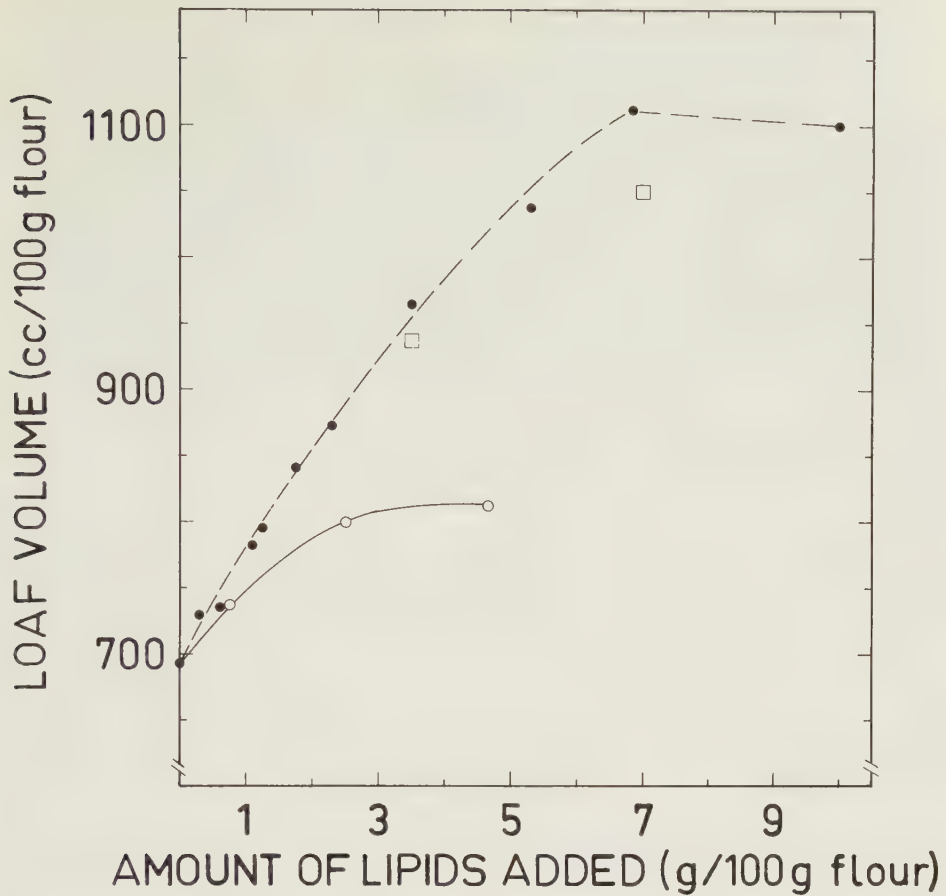


Fig. 28. Effects of loaf volume of additions of lipids to doughs made from the wheat flour mixture. Liposomes made from Epicuron (●). Gel phase made from Dimodan P (□). Liposomes made from Panodan AB 90 (○).

10 times faster to that in the fermentation stage. The stabilization of the surface of the continuous matrix during the rather slow rate of expansion in the fermentation process is not dependent on the presence of polar lipids in a lamellar mesophase, as the fermented volume of doughs made from delipidized flours was the same as that of the original flour (MacRitchie and Gras, 1973). This means that the aqueous phase of the wheat storage proteins also has the ability to form a stable interface during the fermentation. At the fast thermal expansion of the dough volume in the oven, a colloidal stabilizer is needed, which has a structure that can supply material to the interface at the same rate as the surface is expanded. The only structure which has this ability is the lamellar mesophase.



Fig. 29. Bread loaves from the wheat flour mixture with the addition of liposomes from Panodan AB 90. From left to right is added 0, 3.25, and 7.0% of Panodan AB 90 by weight on dry flour.

The large amount of a lecithin mesophase needed in order to obtain the maximum effect on the loaf volume indicates that not only the air/liquid interface is involved in the lipid bilayer stabilization. The properties of the interface between all disperse phases and the continuous matrix will be important for the stability of this matrix towards rupture. The starch granules have dimensions in the range between 1 and 50 μm , and they may therefore make the continuous matrix very thin at certain places, which is illustrated in Fig. 30. Multilayers of a lamellar mesophase forming dispersed particles located at the surfaces of the starch granules and air cells and on other dispersed flour particles will therefore assist in 'healing' ruptures in the foam lamellae and thereby imposing a greater mechanical stability to the continuous matrix.

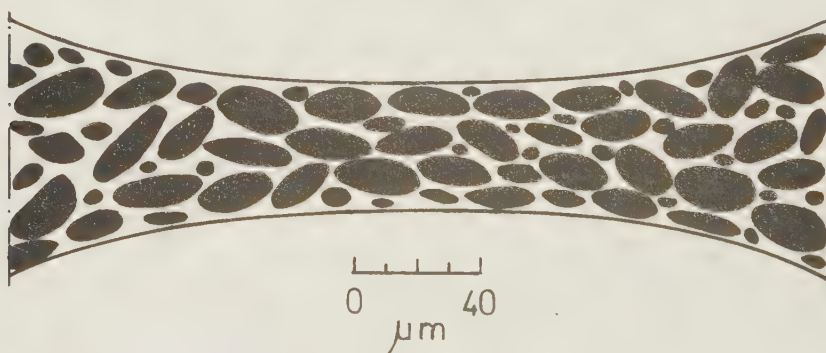


Fig. 30. Schematic illustration of a dough foam lamellae showing starch granules (black) embedded in the continuous matrix. The scale indicated is only approximative.

MacRitchie and Gras (1973) observed that the relation between loaf volume and lipid content showed a minimum at lipid contents intermediate between those of the delipidized and original flour as is shown in Fig. 31. This behaviour may be understood if we recall the behaviour of polar lipids and A-gliadin at the air/liquid interface and accept this system as a relevant model system. The material at the interface will vary between the two extreme states - saturated (i.e. above the collapse point) protein monolayer and saturated polar lipid monolayer corresponding to the presence of no lipids and surplus of lipids. The situation is illustrated schematically in Fig. 32 for a given size of an air/water interfacial area. The mechanical stability of the monolayer with two components is believed to be weaker than for any of the two collapsed states of single components, since the two components are not interacting. The weak part of the mixed monolayer is believed to be the lipid since it is present at a surface pressure where it is more expanded than in its collapsed state. The larger the amount of lipid present at the ESP of the protein the weaker the mechanical stability of the interface

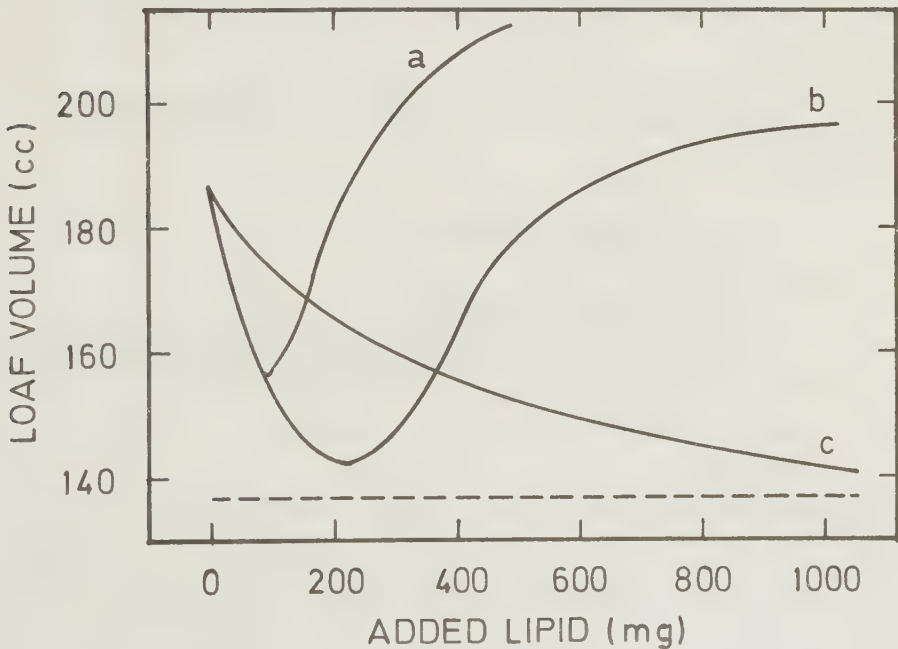


Fig. 31. Effects on loaf volume of additives of a polar lipid fraction from wheat (a), whole wheat lipid (b) and non-polar wheat lipid fraction (c). Dashed line, volume at the end of fermentation stage. Lipid content of the delipidized flour 0.65% (w/w). (MacRitchie and Gras, 1973).

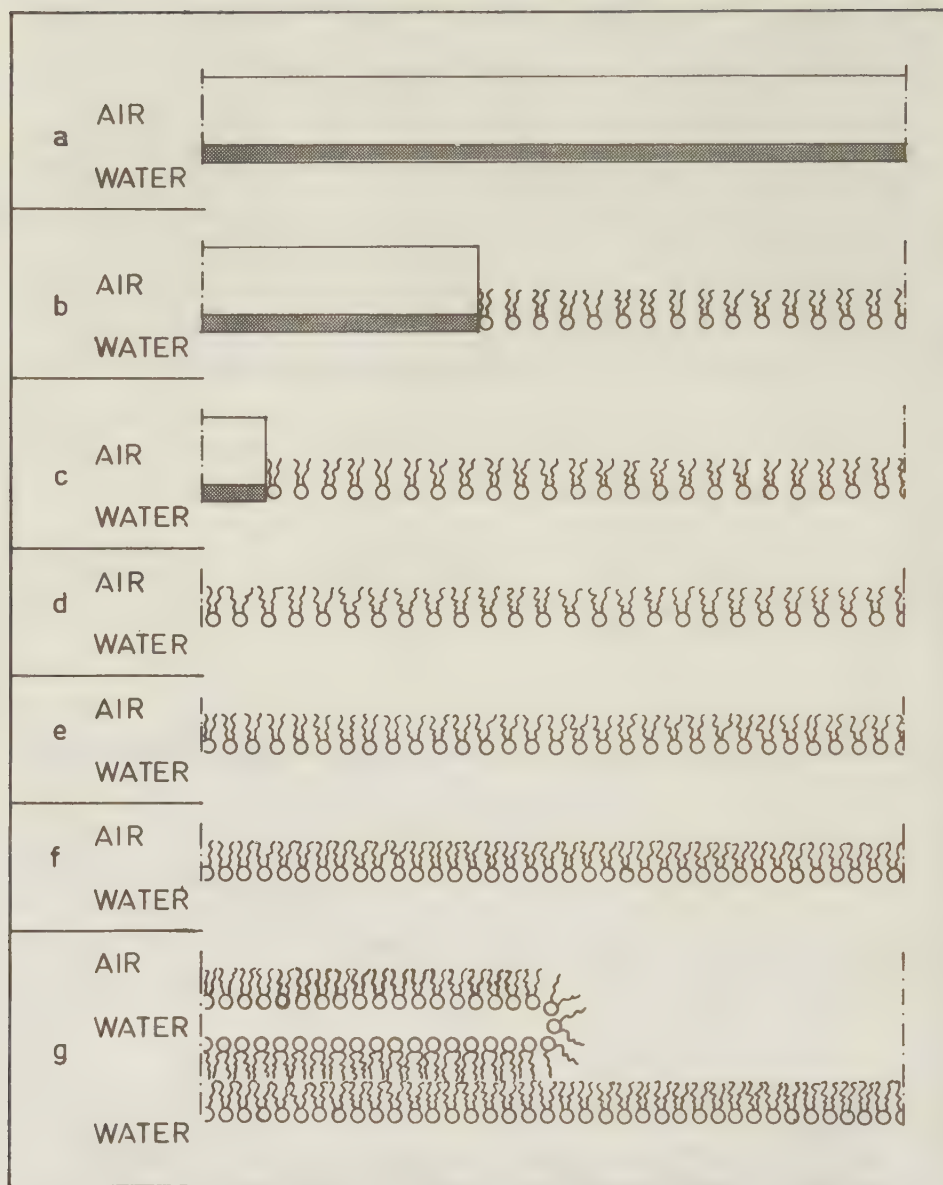


Fig. 32. Illustration of the air/water interface of A-gliadin and digalactosyl diglyceride. In (a) only the collapsed A-gliadin monolayer is present (only one monolayer shown for clarity), to which increasing amounts of digalactosyl diglyceride are added (b to g). In (b), (c) and (d) the lipids are occupying an area ($71 \text{ nm}^2/\text{mol}$), which corresponds to the ESP of the protein. At (d) the proteins are squeezed out of the interface and from here on the lipid molecules will occupy a decreasing area as the lipid content increases, and at (f) the collapse point of the lipid monolayer is reached. Multilayers will form as the amount of lipids increases as is shown in (g).

and the lower the loaf volumes. The mechanical stability of the monolayer is minimal at an amount of lipids, which is large enough to squeeze out the protein monolayer from the interface. This point would be equal to the minimum in Fig. 31. From here on the lipid monolayer will become increasingly more condensed and thereby mechanically stronger as the amount of lipids increases in the system. After the collapse point, bilayers may adsorb to the collapsed lipid monolayer and form a lamellar liquid-crystalline phase at the interface.

Wheat variety. The effect of the PC/water mesophase on the loaf volume is strongly dependent on the flour variety used as is shown in Figs. 33 and 34. The size of the improving effect is dependent on inherent loaf volume capacity of the flours, and the addition of the PC/water phase reveals the loaf volume potential of the flours. The ranking of sizes of the loaf volumes observed, as no PC/water phase has been added, is not representative for the loaf volume capacity of these flours. We believe that this is due to the baking test, which by no means has been optimized for these different flours. By an optimized baking test is meant a baking test in which none of the adjustable variables are limiting (Finney and Barmore, 1948). Adding the PC/water mesophase to the doughs will change the ranking of the loaf volumes for these flours. This result shows that it is important to consider the lipid/water liquid-crystalline phase when making baking tests, as this phase reveals the baking strength of wheat flours without optimizing the baking test for each flour. It should also be mentioned that the appearance of the loaves in terms of crumb texture, porosity and bread shape are improved by the presence of the lamellar liquid-crystalline phase.

5.3 Loaf volumes and flow properties

The loaf volumes, based on the protein contents of the doughs, are shown in Fig. 35, as a function of the rheological parameter $t_{\max.2}$. It is evident from this figure that the size of the loaf volumes, when related to the protein content of the doughs, increases approximately linearly with $t_{\max.2}$. This measure of loaf volume expresses the loaf volume per unit continuous matrix, since the protein content of the dough should be expected to be proportional to the amount of the continuous matrix. Loaf volume per amount of continuous matrix can also be regarded as a measure of the average thickness of the continuous matrix in the dough, if we neglect variations in size distribution and interfacial areas of the air cells between different doughs. $t_{\max.2}$ is the time of the maximum in the relaxation spectrum of the relaxa-

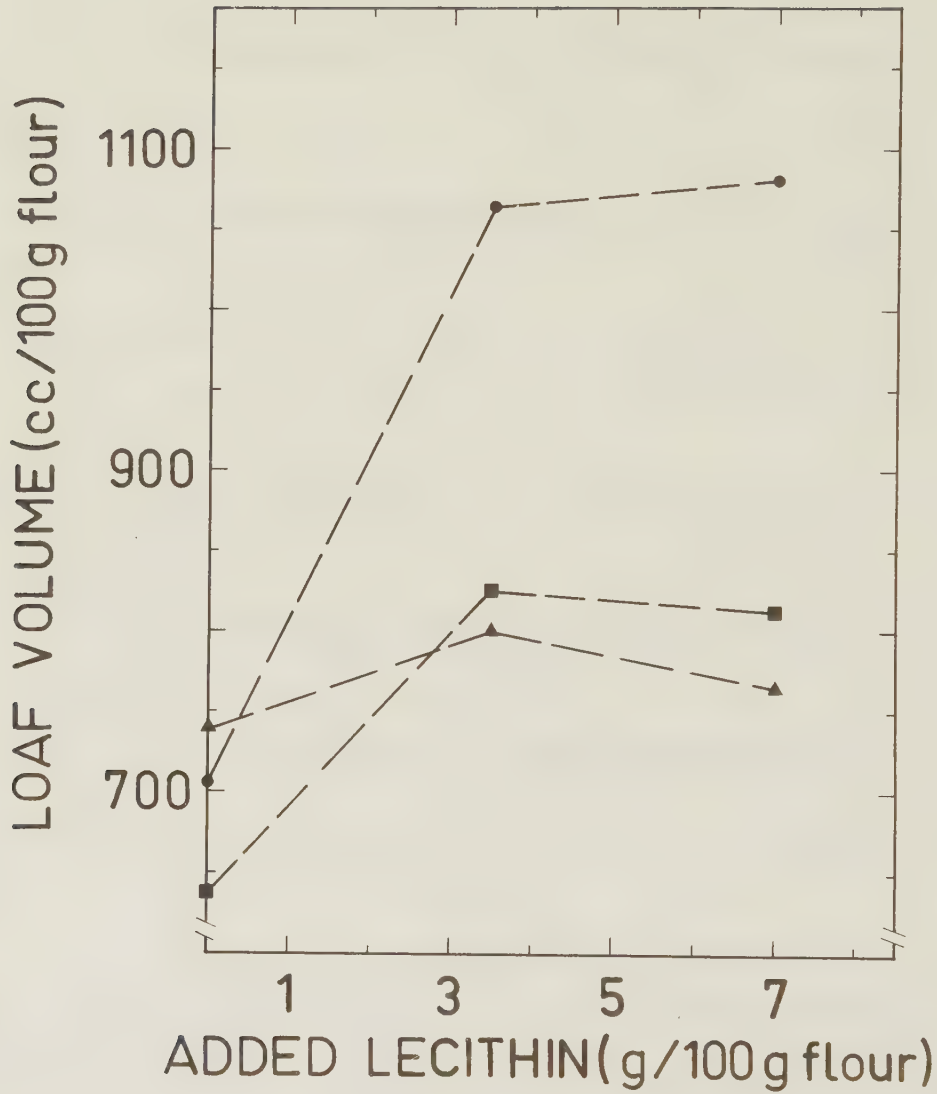


Fig. 33. Effects on loaf volume of the addition of liposomes from Epicuron to doughs made from different wheat flours. Amy (●), Starke II (■), and Maris Huntsman (▲).

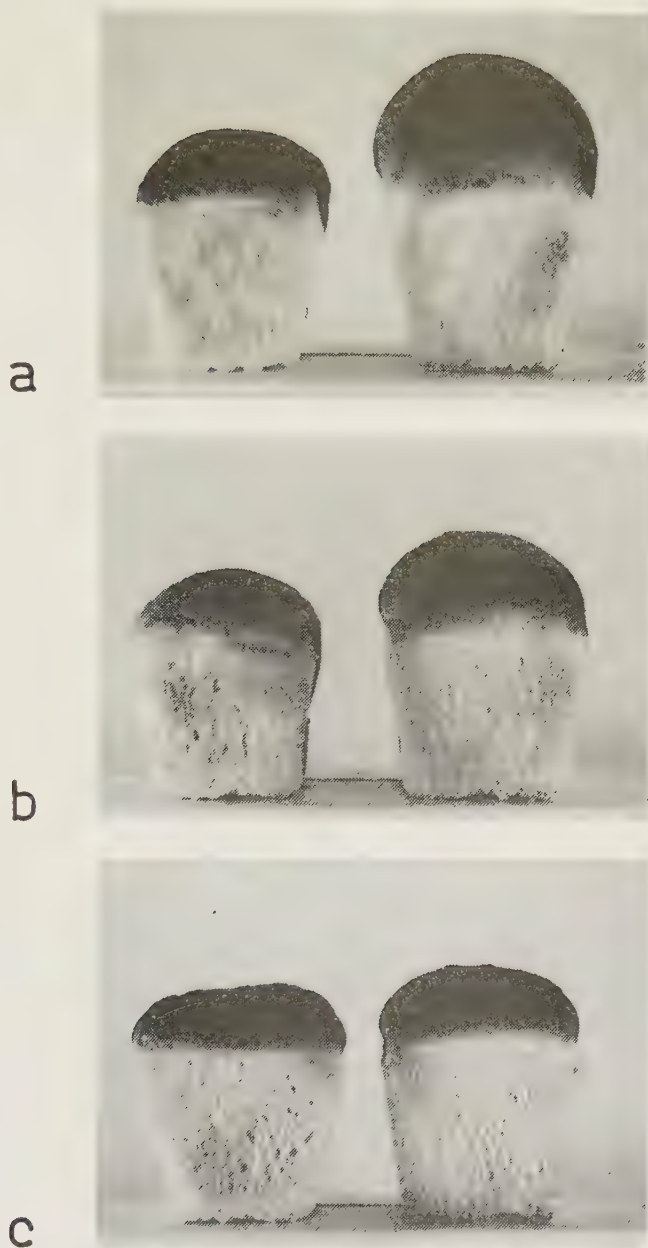


Fig. 34. Bread loaves from a) Amy, b) Starke II, and c) Maris Huntsman with the addition of liposomes from Epicuron. No addition (left) and 3.25% added Epicuron by weight calculated on dry flour (right).

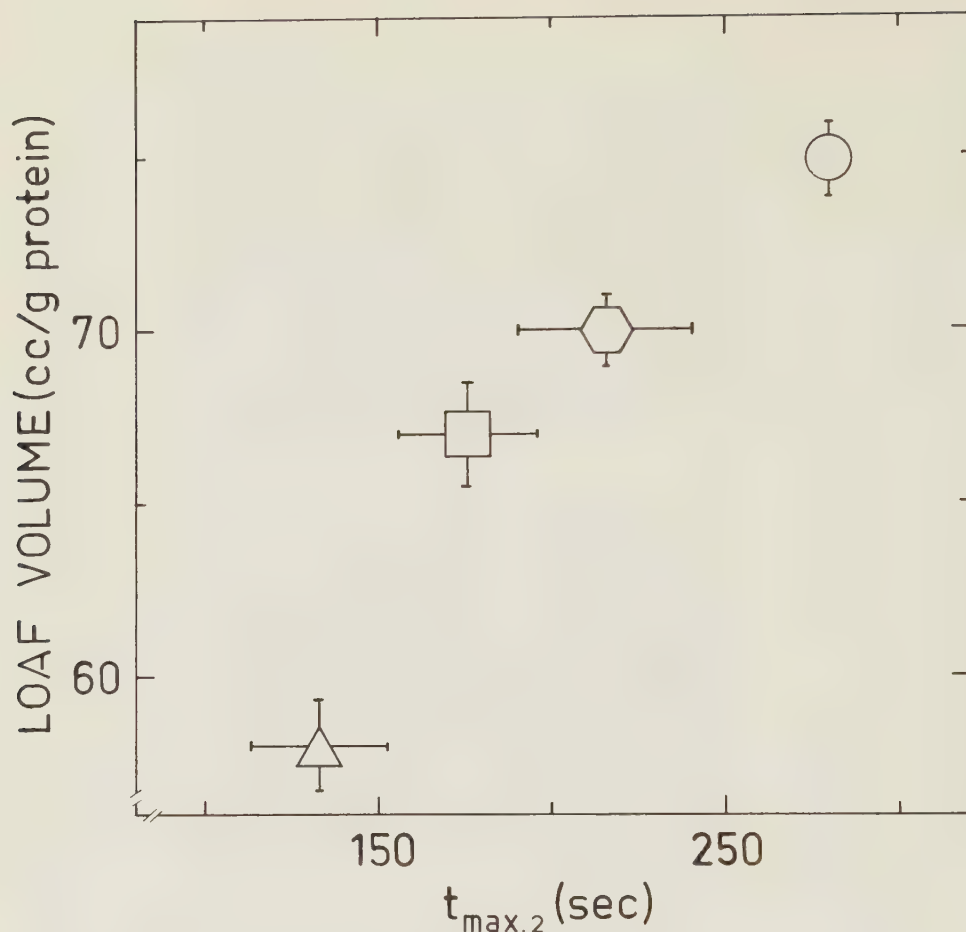


Fig. 35. Loaf volume calculated on protein content basis as function of the time at the maximum of the second relaxation process in gluten ($t_{\max.2}$) for Amy (○), Starke II (□), Maris Huntsman (△), and the wheat flour mixture (○). Error bars show standard deviation from mean value of at least three measurements.

tion process, which is associated with the continuous matrix. The size of $t_{\max.2}$ was interpreted above as an expression of the size and/or relative volume of the 2-coordinated aqueous protein phase in the continuous matrix. The ability of the continuous matrix to form thin stable films should be expected to be dependent on the amount and size of the 2-coordinated continuous phase. The loaf volume per unit protein in the dough should therefore increase with increasing $t_{\max.2}$ and this is also evident from Fig. 35. This is only so if the limiting dough constituent what regards the loaf volume is the same for all the doughs compared. The results in Figs. 33 and 35 indicate that the limiting dough constituent here is the continuous matrix

at optimal amounts of the lipid mesophase. The influence of the starch fraction on loaf volume can be limiting if the α -amylase activity is above the normal value. The influence of high α -amylase activity on the starch fraction is commonly expressed in terms of the falling number (s) (approximately the time taken to liquidify the starch) as measured with a standardized method (AACC, 1973). If the falling number for a flour is below approximately 200 s the loaf volume will be decreased. The loaf volume observed (Fig. 35) for Maris Huntsman could therefore be somewhat diminished due to its low falling number (Table 12).

6 SUMMARY

This work shows that the colloidal properties of a wheat flour dough are relevant to its baking properties. Wheat flour dough is thus described as a colloidal dispersion and several dispersed phases may be identified in its continuous matrix. The most important ones are air cells, lipid particles, yeast cells, starch granules, and other solid particles. We might classify the dough as a gas cell dispersion before its colloidal structure is changed into a foam in the fermentation stage.

The continuous matrix of the dough consists of a concentrated aqueous phase of the wheat storage proteins. These proteins are more amphipathic in their nature than proteins in general and aggregate in water medium, forming a structure proposed to be lamellar. This structure of the wheat storage protein/water phase is supported by rheological results as well as from its optical texture. This structure is the most effective one from a baking functional point of view. The film forming ability of the continuous matrix is one of the most important factors to consider when the dough is processed at the fermentation and baking stages. This property of the continuous matrix is related to a characteristic relaxation time of the continuous matrix, and this time differs significantly for different flour varieties.

The colloidal stability of the continuous matrix is influenced by the presence of the dispersed phases. The lipids are present in dough as dispersed phases. The most important lipid phase in dough from a colloidal point of view is the lamellar liquid-crystalline phase, consisting mainly of polar lipids and water. This lamellar phase can also solubilize non-polar lipids to some extent. The ability of lipids from the wheat varieties, triticale and rye, to form a lamellar liquid-crystalline phase in water differs significantly. The lamellar mesophase is able to stabilize interfaces; the

surface of dispersed phases of air, fat droplets, starch and other solid particles. The stability of the continuous matrix is increased when the lamellar liquid-crystalline phase is present at the interfaces in the dough i.e. thinner films can be produced per unit volume of continuous matrix during the baking process. The loaf volume is therefore increased by the addition of a lipid/water lamellar liquid-crystalline phase to the dough. Crumb texture, porosity and bread form are also improved by this phase.

The size of the increase in loaf volume by the addition of a lamellar meso-phase of lipids is also related to flour variety. The ultimate loaf volume is determined by the ability of the continuous matrix to form thin stable films per unit volume of matrix. This ability is related to the characteristic relaxation time of the continuous matrix, and it is shown that this can be quantified by shear stress relaxation measurements on gluten.

The results presented in this thesis should be of great interest for the wheat breeding, milling and baking industries making possible new approaches both towards the concepts and methods for judging wheat quality.

7 SAMMANFATTNING

Denna avhandling beskriver vetedegen betraktad ur ett kolloidalt perspektiv. Degens kolloidala egenskaper har visat sig mycket betydelsefulla för dess bakningsegenskaper. Vetedegen beskrivs som en kolloidal dispersion, vilken består av en kontinuerlig matrix vari flera diskontinuerliga faser är dispergerade. Den kontinuerliga matrixen består av en koncentrerad vattenfas av vetets förrådsproteiner. Dessa proteiner är mer amfifila till sin natur än vad proteiner i allmänhet är. Vetets förrådsproteiner bildar med vatten aggregat vilka har en struktur som föreslås vara lamellär. Den lamellära strukturen hos protein/vatten aggregaten stöds av resultat och observationer från både reologiska och optiska mätningar. Denna struktur är den mest effektiva med hänsyn till den deformation till tunna filmer som den kontinuerliga matrixen genomgår under bakningsprocessen. Förmågan hos matrixen att utbilda tunna stabila filmer (dess kolloidala stabilitet) är en av de viktigaste faktorerna att beakta vid jäsnings- och avbakningsprocesserna. Denna förmåga är relaterad till en karakteristisk relaxationstid hos matrixen, och denna tid varierar mellan olika mjölsorter. Den kolloidala stabiliteten hos matrixen påverkas av närvaron av de dispersa faserna. Lipiderna förekommer i degen som dispersa faser. Den viktigaste lipidfasen i degen är den lamellära flytande kristallina fasen bestående av i huvudsak polära lipider och vatten. I denna fas löser sig också en del icke-polära lipider. Förmågan hos extraherade lipider från olika vetesorter, råg och tritcale att bilda den lamellära flytande kristallina fasen varierar på ett sätt som korrelerar med förmågan hos dessa spannmål att ge voluminösa bröd. Den lamellära flytande kristallina fasen av lipider och vatten stabiliserar fasgränsytan mellan den kontinuerliga matrixen och dispersa faser av luftceller, fettpartiklar, stärkelse och andra fasta partiklar. Stabiliteten hos den kontinuerliga matrixen ökas när den lamellära flytande kristallina fasen stabiliserar dess fasgränser i degen, dvs tunnare filmer per volymsenhet kontinuerlig matrix kan bildas under bakningsprocessen. Brödvolymer ökar signifikant hos en deg genom tillsats av den lamellära flytande kristallina lipid/vatten fasen till degen. Storleken av volymsökningen hos brödet vid tillsats av den lamellära fasen till degen beror av vetesorten. Den slutliga brödvolymer bestäms av förmågan hos den kontinuerliga matrixen att bilda tunna stabila filmer, då denna volym beror linjärt av den för den kontinuerliga fasen karakteristiska relaxationstiden. Resultaten från denna avhandling bör vara av stort intresse för spannmålsförädlingen, kvarn- och bageriindustrin, då dessa resultat medger både nya begrepp och metoder vid bedömningen av vetets bakkvalitet.

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Finally I want to thank my family and my friends for support and encouragement.

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9 IMPORTANT SYMBOLS AND ABBREVIATIONS

A	average area per molecule or weight substance
CPT	critical peptization temperature
DGDG	digalactosyl diglyceride
ESP	equilibrium spreading pressure
G	shear modulus
$G_{0.3}$	relaxation modulus at 0.3 s
$H(t)$	distribution function of the relaxation spectra
M	molecular weight
M_{app}	apparent molecular weight
M_e	apparent average molecular weight between entanglement coupling junctions
MGDG	monogalactosyl diglyceride
PC	phosphatidyl choline
PE	phosphatidyl ethanolamine
PI	phosphatidyl inositol
PS	phosphatidyl serine
SDS	sodiumdodecylsulfate
SDS-PAGE	gelelectrophoresis performed with sodiumdodecylsulfate in polyacrylamide gel
V	volume fraction
WS	wheat storage
Z	coordination number between cooperative flow units
t	time
$t_{max.2}$	time at the maximum in the plateau zone of the relaxation spectra
$t_{1/2}$	relaxation halflife
v	specific volume
α	a measure of the strength of cooperativity between flow units
γ	shear strain
σ_0	maximum initial stress observed where time is set zero
$\sigma(t)$	stress at time t, time measured from ($\sigma_0, t = 0$)
$(\sigma/\sigma_0)_{1-2}$	relative stress level where the two flow processes intersect or meet
ξ	contiguity parameter
ν	shape factor
Π	surface pressure
τ_m	characteristic relaxation time of the terminal zone

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I

PHASE EQUILIBRIA AND STRUCTURES IN THE AQUEOUS SYSTEM
OF WHEAT LIPIDS

by

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PHASE EQUILIBRIA AND STRUCTURES IN THE AQUEOUS SYSTEM OF WHEAT LIPIDS

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ABSTRACT

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The phase behavior of the aqueous system of lipids extracted from wheat flour (Amy) is described. The phase relations are illustrated in a ternary phase diagram water, polar lipids, nonpolar lipids. The structures have been characterized by X-ray diffraction. At low water content [up to about 15% (w/w)] a liquid crystalline phase of inversed hexagonal type is formed, and at a water content of about 15–50% (w/w) a lamellar liquid crystalline phase exists. A unique feature in this system is

the formation of a liquid lipid-water phase of so-called L2-type. It contains large amounts of water [about 75% (w/w)]. It is proposed that the structure is related to the lamellar liquid crystalline phase by a melting process so that only the long-range order is lost. In addition to these three lipid-water phases there also exists an oil phase (nonpolar lipids) and a water phase in large areas of the phase diagram. The functionality of wheat lipids is discussed in relation to the phase properties.

Wheat-flour contains about 2% (w/w) lipids which are known to have a significant influence on the breadmaking process by interaction with the protein and starch components of the flour (1,2). Numerous studies concern this role of the wheat-flour lipids, often based on lipid extraction of dough and flour (2) or by reconstitution of various extracted lipid fractions with the defatted (or delipidized) flour (3). Knowledge on the structures formed by interaction of the wheat lipids with water is lacking, and the present work concerns the phase behavior and physical structures in the aqueous systems of extracted wheat lipids. From a physical point of view the wheat lipids like all natural lipid mixtures can be separated into a nonpolar fraction, defined as components with no interaction with water, and a polar fraction containing components, which form association structures with water. It is therefore convenient to represent the phase behavior of wheat lipids-water by a ternary system, where the polar and nonpolar components form two of the corners. The structures of the liquid-crystalline phases formed by the interaction of polar lipids with water were first derived by Luzzati and co-workers (4), and the use of ternary phase diagram of this type has been used particularly by Ekwall and co-workers (5). The significance of these properties in food systems has also recently been reviewed (6).

Electron microscopy studies of flour (7) seem to indicate that the lipids of predominately polar type occur in membrane-like aggregates, whereas the lipids of mainly nonpolar type form aggregates resembling oil droplets (8).

The ternary system presented below gives information on possible structures formed when the lipid aggregates in the flour interact with water. The different types of lipid aggregates correspond to different polar-nonpolar ratios in the ternary phase diagram. Knowledge of these ratios and of the amount of water in the dough available for lipid interaction are, however, needed in order to

understand the physical structure of the lipids in the dough and how they interact with proteins and starch.

MATERIALS AND METHODS

An untreated sample of a spring wheat, Amy, from the 1975 crop was experimentally milled in Brabender Quadromat Senior with approximately 65% extraction. The flour contained 13.5% (w/w) proteins ($N \times 5.7$), less than 0.5% ash, and 1.9% crude fat, all calculated on a dry basis. All organic solvents were analytical reagent grades, and solutions were prepared from analytical reagent grade compounds. The reference lipids used were commercial dipalmitoyllecithin and lysolecithin (Sigma) and a synthetic monogalactosyl diglycerides (stearic acid) kindly supplied by the chemical synthesis service of the Swedish Natural Science Research Council. Protein, moisture contents, ash, and crude fat were determined as described in AACC methods 46-11, 44-40, 08-03, and 30-10 (9).

Flour and water saturated *n*-butanol (WSB, volume ratio 20:80) in the ratio of 1:4 (w/w) were mechanically stirred for 150 min at 20°C. The extract was then centrifuged at $1800 \times g$ for 60 min. The supernatant was collected and solvents were removed in a rotating evaporator under vacuum below 45°C. Ethyl-ether was used to dissolve the lipids in the residue and the ether was evaporated using a stream of nitrogen. The lipid residue was finally dried over P_2O_5 for 24 hr and stored at -18°C. The amount of lipids was determined gravimetrically.

The crude lipid mixture obtained by extraction was separated according to MacMurray and Morrison (10) into nonpolar and polar lipids. A simpler and faster method (11) was used in order to obtain polar lipids in amounts sufficient for phase studies. The method was modified with a fourth elution step using acetone to obtain monogalactosyl diglyceride, as the method originally (11) was developed for separation of phospholipids.

Thin-layer chromatography (tlc) analysis was done using plates with 0.25 mm silica gel (Merck). The solvent system was chloroform:methanol:water 65:25:4 (v/v/v) and the plates were sprayed with a solution of 30.0 g copper acetate in 92.2 g 85% phosphoric acid and heated for 30 min at 180°C.

The lipid-water phases were prepared by mixing water and the extracted lipids in ratios differing by 5 or 10% (w/w) in composition. Each sample was mixed and kept at room temperature during about 24 hr in order to reach equilibrium, and then centrifuged at $40,000 \times g$ for 2 hr. The water content of the phases was determined by the Karl-Fischer method. The different phases were then examined in the polarizing microscope and characterized by X-ray low-angle diffraction using a Guiner type of camera, as described by Larsson (12).

RESULTS AND DISCUSSION

Lipid Separation

Eighty-eight percent of the crude fat was extractable with WSB. Of this lipid mixture 55% (w/w) were polar lipids and 45% were nonpolar separated as described by MacMurray and Morrison (10). In the faster separation method (11) the first and the fourth eluates were taken as polar lipids and the second and the third as nonpolar lipids. The method by MacMurray and Morrison (10) is

based on elution by hexane/ethyl ether/ether/chloroform/acetone/methanol, whereas the method by Sen Gupta (11) uses light petroleum/ethylether/acetone. The ratio polar/nonpolar lipids obtained in this way differed by less than 1% from values obtained by the method by MacMurray and Morrison (10) (*i.e.*, below the experimental errors). The overall recovery from the column was 98%. A tlc-pattern shown in Fig. 1 illustrates the resolution of this simpler separation method.

A small amount of protein, which also is obtained by this lipid extraction, appeared to have no influence on the phase properties. Protein and contaminating starch granules can be separated by ultracentrifugation.

Phase Behavior

The phase diagram of the ternary system polar wheat lipids, nonpolar wheat lipids and water is shown in Fig. 2. Different proportions of polar/nonpolar lipids obtained by chromatographic separation or by separation of well-defined phases of Fig. 2 were mixed with water, and the phases formed were analyzed as described above. At all compositions above 5% (w/w) of water a liquid oil phase, free from polar lipid components according to tlc, is formed together with other

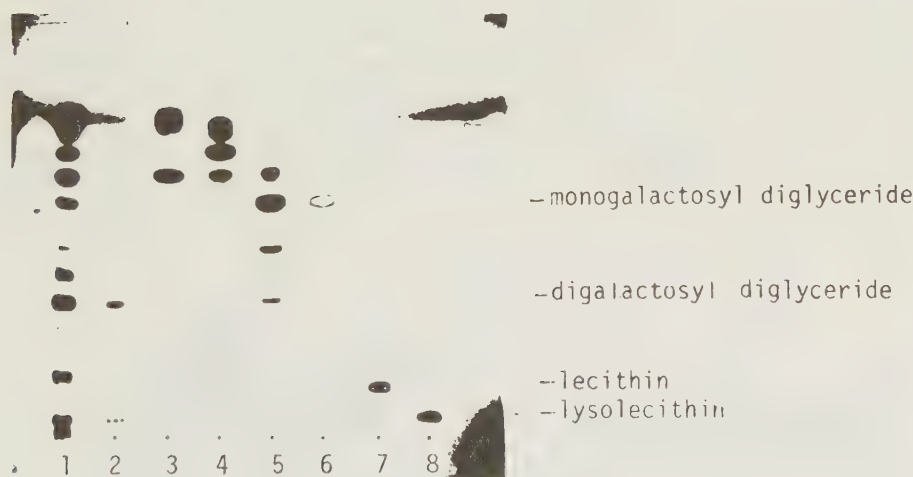


Fig. 1. TLC-pattern of the liquid phase L2 containing about 10% (w/w) nonpolar and 90% polar lipids. From left to right is shown the total lipids, the first fraction in light petroleum (40° – 60° C), the second fraction in light petroleum - ethyl ether 87:13 (v/v), the third fraction in ethyl ether, the fourth fraction in acetone, and the standards monogalactosyl distearin, dipalmitoyl lecithin and lysopalmitoyl lecithin. The solvent system used was chloroform-methanol-water (65:25:4). The spots from the reference lipids are weak due to their saturated character and have therefore been indicated by black pencil. The first fraction is dominated by digalactosyl diglycerides, the second by triglycerides and fatty acids, the third by sterols and the fourth by monogalactosyl diglycerides.

phases. Four types of phases dominate the phase diagram. Besides an oil and a water phase, two types of lipid-water phases are formed. One is a liquid phase which has the characteristics of a so-called L2-phase (5) and the other is of liquid/crystalline character (4). As shown in Fig. 2 the extracted lipids have a polar/nonpolar ratio of 55:45 and the broken line from this point to the water corner represent the binary system formed by wheat lipids and water.

All compositions of water added to total extracted lipids caused a nonpolar oil phase to separate on top of the samples. At low-water content a hexagonal liquid crystalline phase is formed, which at higher water content is transformed into the lamellar liquid crystalline phase. The structure of the two liquid-crystalline phase is illustrated in Fig. 3.

The hexagonal spacings, equal to the distance between the center of the water cylinders, were found to be 70.4 and 73.2 Å when water was added to the total

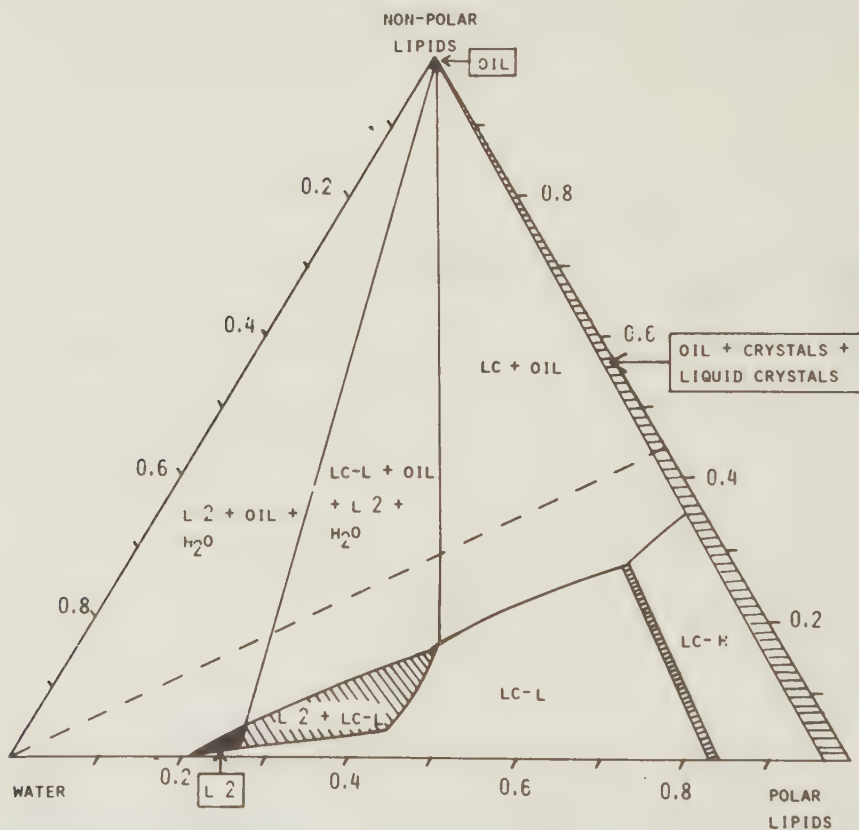


Fig. 2. Ternary phase diagram of wheat lipids and water at 25°C represented by the polar and nonpolar lipid fractions as two of the components. The hexagonal and lamellar liquid crystalline phases are called LC-H and LC-L, respectively. The broken line corresponds to the binary system of the total extracted lipids and water.

lipids to a water content of 6 and 12% (w/w), respectively. These parameters are reasonable in comparison with the lamellar spacings given below.

The lamellar liquid crystalline phase exists over a wide composition range, and at high water content there is a successive transition to a liquid phase (L2) described below. The swelling properties of the lamellar liquid crystalline phase is shown in Fig. 4. When this phase is formed by the total extracted lipids and water, the thickness of the lipid bilayer (obtained by extrapolation to a water content of zero) is 57 Å. The ratio of nonpolar/polar lipids in this phase is about 35:65 (w/w). When the lamellar phase is enriched into polar lipids to a ratio nonpolar/polar of about 20:80, the repetition period of the lamellar phase is reduced, and the lipid bilayer thickness is 49 Å. The spacings of the lamellar phase formed by only polar lipids correspond to a lipid bilayer thickness of 40 Å. These differences show that the triglyceride molecules of the nonpolar lipids solubilized in this phase, to a large extent must be accommodated in the gap between the lipid bilayer, and not arranged with their chains along the chains of the polar lipid molecules. In the case of lipid fractions with a nonpolar/polar ratio of 20:80 it was possible to record sharp diffraction lines corresponding to spacings up to 124 Å. The lamellar liquid crystalline phase exists up to very high water contents but the diffraction lines are successively broadened and hard to measure due to reduced domain size of this phase, which is related to the presence of the liquid phase (L2), as will be further described.

Relative to this, the aqueous system of galactolipids has been studied by Shipley *et al.* (13), who found that a monogalactosyl diglyceride forms only a hexagonal liquid-crystalline phase with water of the same type as described here, whereas the corresponding digalactosyl diglyceride forms a lamellar liquid-crystalline phase. They both exist in the composition range of about 10–20% (w/w) of water. It is also known that various phospholipids form a lamellar liquid-crystalline phase with water (4), whereas there are no studies of mixed

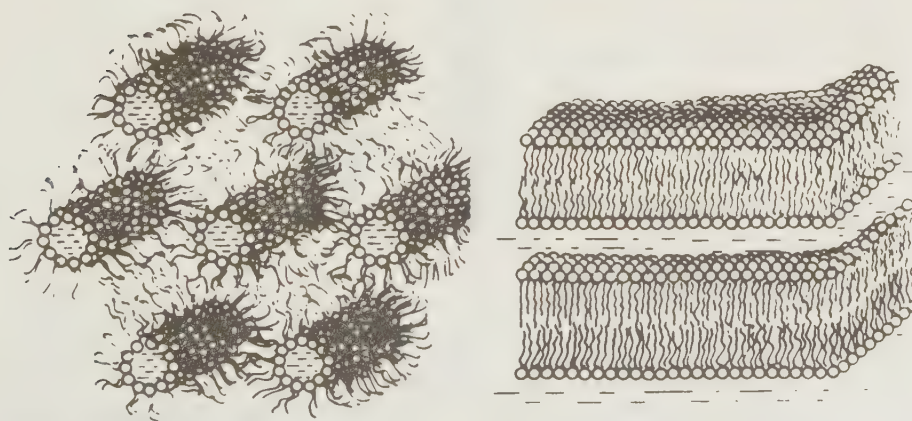


Fig. 3. Illustration of the structure of the lamellar liquid crystalline phase (left) and the hexagonal liquid crystalline phase (right) occurring in aqueous systems of wheat lipids.

systems of galactolipids and phospholipids, such as the polar fraction of wheat lipids.

The most remarkable feature of this phase diagram is the existence of an L2-phase formed in excess of water. It is an optically isotropic liquid with the appearance of a clear yellow oil, and contains about 75% (w/w) of water. It exists only in the presence of a water phase, and since it is heavier than water, it is separated from the triglyceride oil phase by a water layer (Fig. 5). The L2-phase of this system is unique in many respects when compared with L2-phases described earlier by other authors, thus it is discussed in detail.

The so-called L2-phase was first described by Fontell and co-workers (14) in the ternary system sodium caprylate:decanol:water as an amphiphile in the liquid state containing water in the form of inversed micelles (L1 is the ordinary micellar water solution). It has later been observed in numerous other amphiphile-water systems (5). The detailed structure is not known. On the basis of an X-ray scattering analysis of this phase in binary system, particularly the swelling in relation to the lamellar liquid crystalline phase, it was proposed that it possesses the same gross structure as liquid crystalline phases, except that the

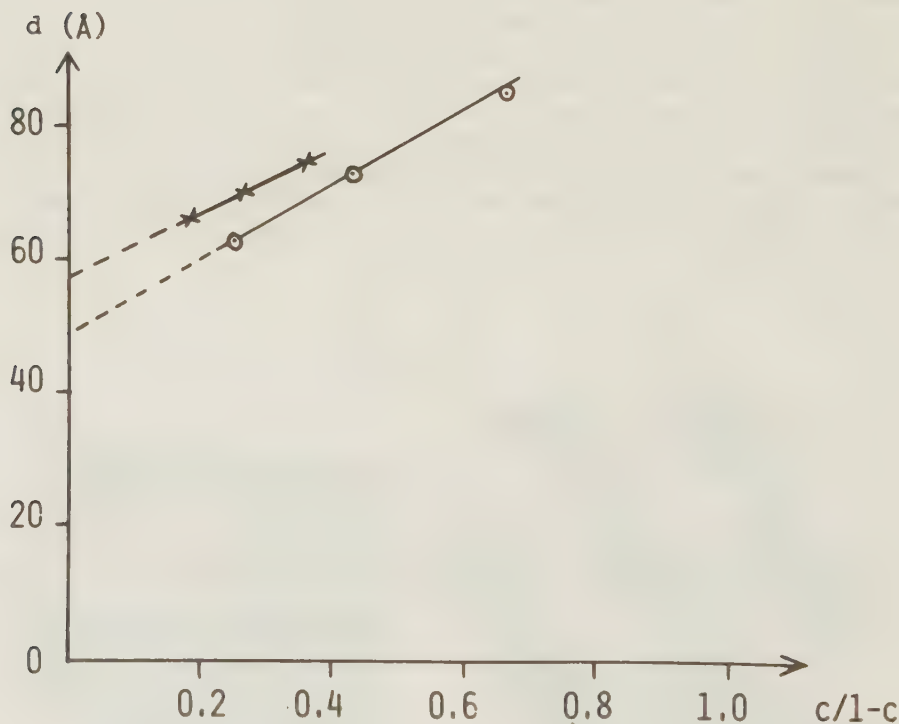


Fig. 4. Lamellar unit distance obtained by X-ray diffraction of the lamellar liquid crystalline phase as a function of c , $L - c$, where c is the weight fraction of water. Crosses represent samples obtained when water is added to the total lipids, and circles correspond to a polar-enriched lipid fraction, where the polar:nonpolar ratio is about 80:20.

long-range order is lost (15).

It is thus formed by melting of the lamellar or inversed hexagonal liquid-crystalline phases, and the X-ray data indicate that the structural units are the same. In the case of an L2-phase formed by melting the lamellar liquid crystal of monoglyceride-water phases, the size of the lamellar units was estimated to be about 300 Å; *i.e.*, water occurs as lamellar disks with this diameter in a lipid environment (12,15). In the present system the L2-phase is formed from the lamellar liquid crystalline phase by heating, and the L2-phase is, therefore, proposed to consist of lamellar units, *i.e.*, the same type of structure as in monoglyceride-water systems (15).

The L2-phase has not previously been observed in aqueous systems of any natural lipids. Its formation in wheat lipids is probably a consequence of disorder due to the high water content, the presence of nonpolar lipids (for the nonpolar/polar lipid ratio of this phase see Fig. 1), and the high degree of unsaturation of hydrocarbon chains (10), so that the 'melting point' of the lamellar liquid crystalline phase is lowered. The amount of lysolecithin in the lipid extract is an important factor for formation of this phase.

According to the general behavior of lipid-water systems, the addition of water to the lamellar liquid crystalline phase above its limit of swelling can result in

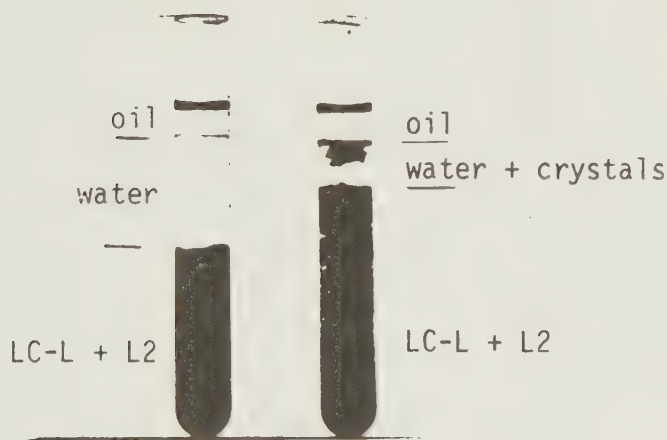


Fig. 5. Samples of lipids from Amy (right) and Maris Huntsman (left) equilibrated with 70% (w/w) of water and separated by ultracentrifugation ($300,000 \times g$ for 2 hr). Under the oil and water phases there is first a region of lamellar liquid crystals dispersed in the L2-phase (see Fig. 7) and under this region there is a pure L2-phase. Some lipid crystals can also be seen in the water phase formed by Amy lipids, but this is only a consequence of the strong sedimentation gradient.

three alternative phase changes. The first is the formation of a hexagonal phase in the case of lipids with micellar solubility in water, which is not the case in the present system (4). The second and most common is the formation of a dispersion of closed particles with spherically concentric lipid bilayers alternating with water layers, so-called 'liposomes' (12). The third alternative corresponds to temperatures above the 'melting point' of the liposomes, when an L2-phase is formed in equilibrium with water, as observed in the wheat lipid-water system. These phase relations are illustrated in Fig. 6 in the case of two simple aqueous

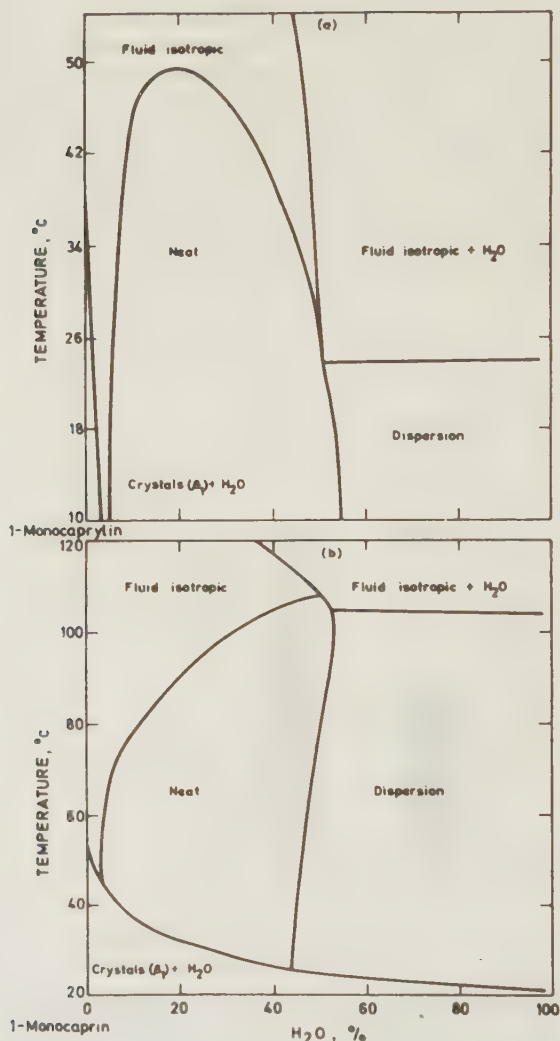


Fig. 6. Binary system of 1-monocaprylin-water and 1-monodecanon-water (12) illustrating the relation between the L2-phase (denoted fluid isotropic in the diagram) and the lamellar liquid crystalline phase.

systems of monoglycerides. Above 24°C 1-monocaprylin forms an L2-phase in equilibrium with water. In the phase diagram of the wheat lipids (Fig. 2) there is a broad region where the L2-phase and the lamellar liquid crystalline phase coexist. This is an anomaly not in agreement with a simple melting behavior, and indicates that there is segregation of the polar lipid molecules so that the ones with higher 'melting point' are enriched in the liquid crystalline phase. This region of the phase diagram is also the only one which gives deviations from the phase rule applied to a three-component system. Thus there is a large four-component triangle (Fig. 2) from this region toward the oil corner, which shows that the system behaves as consisting of at least four components, and the polar lipids are therefore expected to deviate from the simple one-component behavior in this region.

The existence of lamellar phases with a water layer thickness above about 20 Å is due to the presence of charged lipids (4,16). The existence range of the L2-phase with as high water content as observed in the present system should therefore be expected to be sensitive for the amount of charged lipids. It is known that different types of lipids occur in about the same relative amounts in wheat (10), but very small chemical differences, particularly with regard to proportion of ionic lipids, might result in formation of different association structures. We have compared the lipids from the cultivar Maris Huntsman (poorer baking properties than Amy) with those from Amy in order to check this, and the result is shown in Fig. 5. With 70% (w/w) of water added it is obvious by visual comparison that there are pronounced differences. The lipids from Amy give a dominating L2-phase (70% w/w) with water (17% w/w) and nonpolar oil (13% w/w), whereas lipids from Maris Huntsman give a dispersion of liquid crystalline phase in L2, about the same relative amount of oil and about double the relative amount of water.

As suggested the L2-phase can be regarded as a melt, and crystallization is achieved by cooling or by reduction of water content. The crystallization has been studied in the polarizing microscope. The lamellar liquid crystalline phase is always obtained as spherical droplets dispersed in the L2-phase. As seen in Fig. 7 it shows the same optical texture as the dispersion formed by the lamellar liquid crystal in excess of water (12). This shows that the lamellar liquid crystalline phase dispersed in the L2-phase has the same type of closed structure as the 'liposomes', *e.g.*, particles with spherically concentric lipid bilayers alternating with water layers. In order to fit with the suggested structure of the L2-phase, with water disks in a hydrocarbon chain matrix, the surface of the 'liposomes' in the present system should, however, be expected to consist of hydrocarbon chain tails. In this way the interfacial energy between the L2-phase and the liquid-crystalline particles will be extremely low, which should explain why the particles form very stable 'micro dispersions' with no tendency to aggregate and separate, even at centrifugation at $200,000 \times g$ for 2 hr. This shows also that the density of these two phases must be almost identical, which is consistent with the structural relations proposed here.

X-Ray diffraction studies of lipids extracted from wheat flour have been reported previously by Traub *et al.* (18) and Grosskreutz (19). Differences in these data might be due to different degree of water swelling or even different phases, as the general structural features of lipid-water systems were not known at that time.

On the Functionality of Wheat Lipids

Lipids extractable from flour in dough with light petroleum and WSB are classified as 'free' and 'bound', respectively. The term 'free' and 'bound' lipids, based only on extraction behavior, need not have any true relation with the association of the lipids with other flour components.

It has been observed that the amount of 'free' lipids is reduced if the flour is treated with water to give a dough (1). This is not surprising with regard to the phase properties evident from the present work. When water is added, the liquid crystalline phases formed will solubilize nonpolar lipids, and the ordered association structure, like the lamellar phase with hydrophilic interfaces, will protect the nonpolar lipids against nonpolar solvents.

In a recent work on the effect of sucrose esters in breadmaking (20) it was found that the effect increased with the polarity of the lipid, and the best effect was obtained when the HLB-value (hydrophilic/lipophilic balance) was as high as that of diagalactosyl diglycerides.¹ Again, the liquid crystalline phase formed by digalactosyl diglycerides is of lamellar type, where the less polar monogalactosyl diglycerides give a hexagonal liquid crystalline phase.

¹Recent published work by F. MacRitchie (J. Sci. Food Agr. 28: 53, 1977) supports these findings.

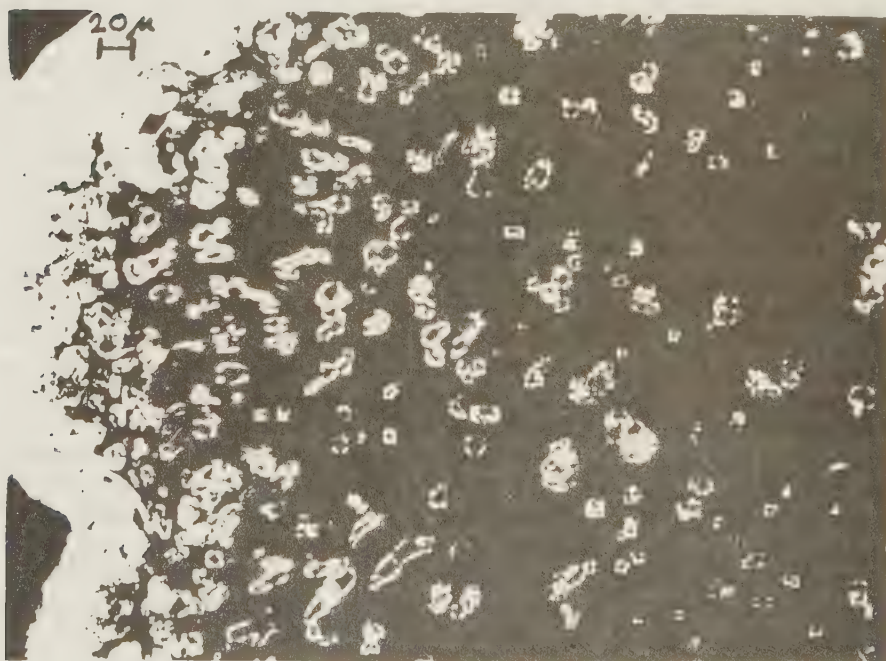


Fig. 7. Formation of the lamellar liquid crystalline phase in the form of closed spherically concentric particles dispersed in the L₂-phase as seen in the polarizing microscope. The border to the left is continuous liquid crystalline region.

The lamellar and hexagonal liquid crystalline phases shown in Fig. 3 have quite different rheological properties. The hexagonal phase forms aggregates uninfluenced by excess water or other phases present in the system, whereas the lamellar phase forms multilamellar films at the oil/water, air/water (6) or starch/water (21) interfaces. The L2-phase might also have functional effects. An interesting property of this phase is the liquid state, a unique feature which should be very favorable for diffusion to other components.

The effect of different nonpolar wheat lipids was recently reported (22) by a study of the breadmaking when these lipids were added to flour and defatted flour. Fatty acids were found to give a pronounced negative effect, and of different fatty acids linoleic acid was dominating. A reason for this might be that fatty acids added to a lamellar liquid crystalline phase have a tendency to change the structure to hexagonal. We have found that fatty acids added to lamellar liquid crystalline phases of monoglycerides give hexagonal phases, and that fatty acids are more effective in this respect than triglycerides. This is probably due to a higher tendency of fatty acids to be solubilized in the lipid bilayers of the liquid crystalline phase, whereas triglycerides tend to form a separate oil phase. Furthermore the tendency to form hexagonal phases increases with increasing amount of *cis*-double bounds in the chains.

Phase studies of lipids from other cereals are currently in progress.

Acknowledgments

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PHASE EQUILIBRIA IN THE AQUEOUS SYSTEM OF WHEAT GLUTEN
LIPIDS AND IN THE AQUEOUS SALT SYSTEM OF WHEAT LIPIDS

by

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Phase Equilibria in the Aqueous System of Wheat Gluten Lipids and in the Aqueous Salt System of Wheat Lipids

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ABSTRACT

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The aqueous system of gluten lipids was studied for comparison with the wheat lipid-water system. Phase equilibria were determined and were illustrated in the form of a ternary system: nonpolar lipids/water/polar lipids. The structures of the phases were characterized by x-ray diffraction. Besides water and the nonpolar oil phase, three aqueous phases were observed. The lamellar phase and the reversed hexagonal liquid-crystalline phase were frequently observed in aqueous systems of polar lipids. A third liquid phase containing about 50–80% (w/w) water is analogous to the so-called L2-phase in simple amphiphile-water systems. The water system of wheat lipids is the first system of biological origin found to exhibit this

phase. The functionality of the different phases with regard to baking is discussed on the basis of the phase properties. The physical behavior of extracted wheat lipids in the presence of a salt solution corresponding to that in a dough (0.260M NaCl, 0.070M KCl, 0.016M MgCl₂, and 0.004M CaCl₂) also was analyzed and related to phase equilibria and to corresponding structures in the aqueous system of wheat lipids. The degree of solubilization of nonpolar lipids in the lipid-water phases was substantially reduced when salt was present, and the phase equilibria involving the L2-phase was different.

Phase equilibria and structures in the aqueous system of wheat lipids were recently reported (Carlson et al 1978). The phase behavior was described by a ternary phase diagram: water/polar lipids/nonpolar lipids. To obtain as much of the lipids as possible, they were extracted from wheat flour by water saturated n-butanol (WSB). To differentiate between the behavior of the total lipids obtained in this way and that of the lipids associated with the gluten fraction, we have similarly analyzed the aqueous system of lipids extracted from gluten.

The phase properties in lipid-water systems are well-known to be sensitive to the presence of electrolytes, particularly when the lipids contain ionic components (Larsson and Lundström 1976). Because a dough contains ions, the interaction between wheat lipids and salt solution is of interest. Within the lipids, the cations are of particular interest as counter-ions because all charged lipid species are anionic (Morrison et al 1975). As in our study of the wheat lipid aqueous system, WSB was used as solvent.

MATERIALS AND METHODS

The flour used was a Swedish spring wheat (AMY 1975), the same batch as studied earlier (Carlson et al 1978). Gluten was prepared with a semi-automatic gluten washer (Falling Number) and was used immediately. Wet gluten (67% [w/w] water) and 99% (w/w) ethanol were mixed to give a concentration of approximately 95% (w/w) ethanol in the gluten-solvent mixture. The mixture was homogenized in a Sorvall Omni-Mixer and stirred during extraction for 4 hr at 20°C. Because use of WSB in the gluten extraction resulted in formation of a stiff gel that was hard to handle in a reproducible way, ethanol was used. The extract was then centrifuged at 30,000 × g for 2 hr. The solvent was removed from the supernatant by a rotary vacuum evaporation at a temperature below 45°C. The collected lipid extract was dried over P₂O₅ for 24 hr before weighing and was stored at -18°C before use. The extraction was repeated twice. Aqueous samples were prepared immediately. Even after several weeks of storage, phase behavior did not differ. The flour was extracted as in the previous study (Carlson et al 1978).

Details concerning determination of the ratio of polar to nonpolar lipids, thin layer chromatography (TLC), preparation of lipid-water phases, and structure analysis of the different phases obtained were reported previously (Carlson et al 1978).

The salt solution (0.260M NaCl, 0.070M KCl, 0.016M MgCl₂, and 0.004M CaCl₂) was calculated to correspond to the salt

concentration in a dough. The calculations are based on mineral amounts given for HRW-flour (Toefer et al 1972) and on a concentration of 0.25M NaCl in the dough-liquor. The pH of the salt solution was approximately 5.

RESULTS AND DISCUSSION

Lipid Separation

The extractable lipid content of dry gluten was 6.8% (w/w). The lipid extract contained equal amounts of polar and nonpolar lipids. A TLC-pattern of the lipids extracted from flour and gluten is shown in Fig. 1.

Phase Behavior of Gluten Lipids-Water

The main features of the phase diagram for gluten lipids-water, illustrated in terms of three components (Fig. 2), are similar to those of the phase diagram of flour lipids-water (Carlson et al 1978). The principal phases were water, oil (nonpolar), and lipid-water phases. Two types of lipid-water phases were formed: a liquid phase with characteristics of an L2-phase and a liquid-crystalline phase. The liquid-crystalline phase consisted of a hexagonal liquid-crystalline phase of reversed type and a lamellar liquid-crystalline phase. The broken line in the phase diagram represents the binary system formed by gluten lipids and water.

Nonpolar lipids separated as a liquid oil phase on top of all sample compositions above 5% (w/w) water. At water contents up to about 15% (w/w), a reversed hexagonal liquid-crystalline phase was formed. At a water content of 15–40%, a lamellar liquid crystalline phase existed, which transformed into an L2-phase as the amount of water increased.

The significant differences between the phase behavior of gluten lipid-water systems (Fig. 2) and of wheat lipid-water systems (Fig. 3) were:

- 1) In the lamellar liquid-crystalline phase region of the gluten lipids-water system (Fig. 2), solubilization of nonpolar lipids increased as the water content increased, whereas in the same phase of the wheat lipid-water system the opposite was observed (Fig. 3).
- 2) The four-phase area, which deviated from the phase rule applied to the three-component system, was smaller in the gluten lipid-water system (Fig. 2) than in the wheat lipid-water system (Fig. 3). In the wheat system, this region disappeared as salt was included in the water, as described below.
- 3) The L2-phase existed up to 87% of water in the gluten lipid-water system (Fig. 2), whereas its maximum water content was about 78% in the wheat lipid-water system (Fig. 3). The two-phase region containing L2 and lamellar phases was also smaller in the gluten system. The L2-phase often contained small amounts of

liquid-crystalline phase, however, and the border lines between these phases were therefore hard to establish.

Functionality of Lipids in Gluten

The existence of gluten depends on both its protein and its lipids. The state of association of the gluten lipids in gluten is not known. Different lipid-water phases have drastically different functional properties. What functional properties of gluten can be attributed to the lipids? The water distribution between the components in a dough is not known, and therefore the lipid phase that is formed is not known. If we consider the different possible phases, however, the lamellar liquid-crystalline phase would be expected to contribute to baking properties. The most important property of

this phase is its surface activity; it can form either hydrophobic or hydrophilic interfaces according to the environment. The lamellar liquid-crystalline phase is, therefore, very efficient as a dispersing and lubricating agent, in contrast to the hexagonal liquid-crystalline phase and the L2-phase, which in these respects behave like a plastic fat and a nonpolar oil, respectively.

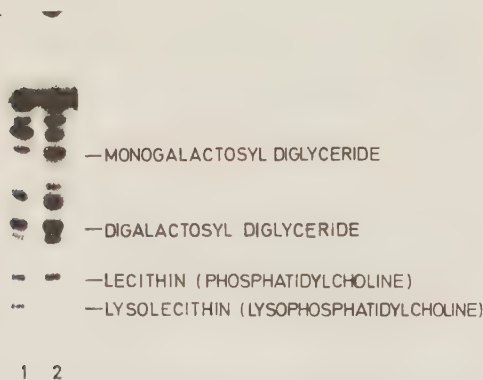


Fig. 1. TLC-pattern of wheat lipids. 1, Water saturated *n*-butanol extract from AMY flour. 2, 95% ethanol extract from AMY-gluten. The solvent system used chloroform-methanol-water (65:25:4); 200 μ g lipids were applied from each extract.

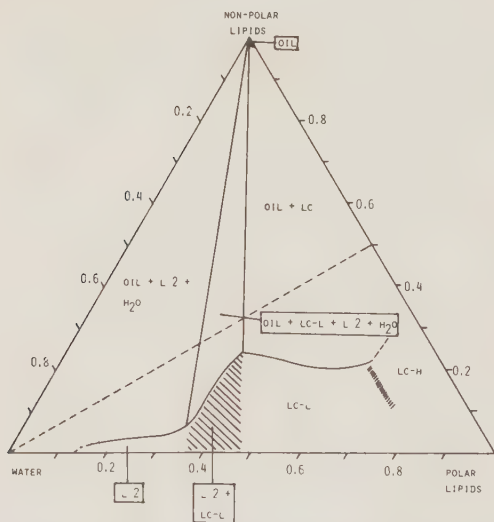


Fig. 2. Ternary phase diagram of gluten lipids at 25°C represented by water, polar, and nonpolar lipids. LC = liquid-crystalline phase. LC-L = lamellar liquid-crystalline phase. LC-H = reversed hexagonal liquid-crystalline phase. The broken line corresponds to the binary system of the total extracted lipids and water.

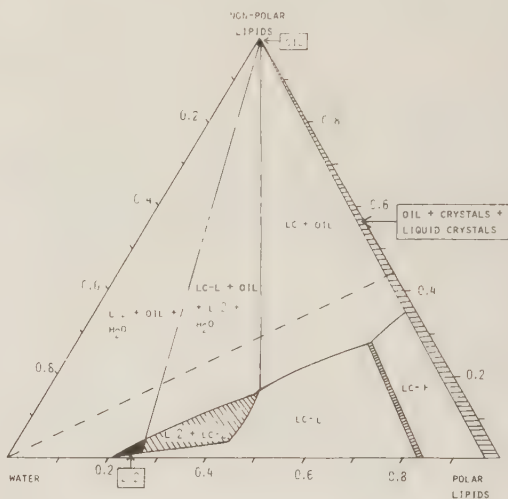


Fig. 3. Phase diagram of wheat lipids and water at 25°C from previous study (Carlson et al 1978). LC = liquid-crystalline phase. LC-L = lamellar liquid-crystalline phase. LC-H = reversed hexagonal liquid-crystalline phase. The broken line corresponds to the binary system of the total extracted lipids and water.

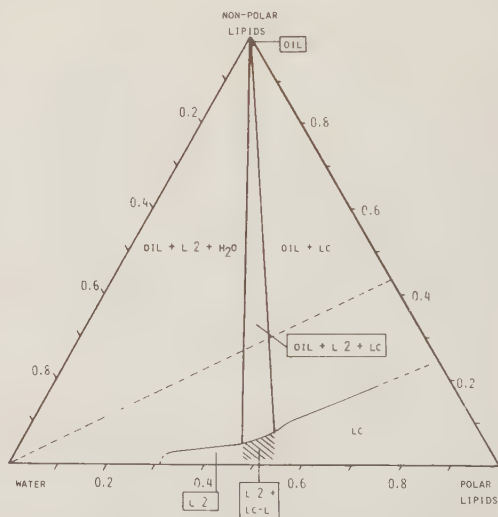


Fig. 4. Ternary phase diagram of wheat lipids and an aqueous salt solution at 25°C. L = liquid-crystalline phase. LC-L = lamellar liquid-crystalline phase. LC-H = reversed hexagonal liquid-crystalline phase. The broken line corresponds to the binary system of the total extracted lipids and water.

Wheat Lipids and Aqueous Salt Solution

As defined by a ternary system, the phase behavior of the aqueous salt solution and the extracted wheat lipids, divided into polar and nonpolar components, is given in Fig. 4. When this phase diagram is compared with that of the aqueous system (Fig. 3), the most striking effect of the presence of ions is the reduction of the degree of solubilization of nonpolar lipids in the liquid-crystalline phase and in the L2-phase. The lamellar liquid-crystalline phase exhibited a one-dimensional swelling to a maximum lamellar thickness of 73 Å at a water content of about 40% (w/w). The value of the lipid bilayer thickness of this phase, obtained by extrapolation to a water content of zero, is 42 Å, which is much smaller than the corresponding bilayer of the saltfree system (Carlson et al 1978). The salt-containing system apparently accommodates the triglyceride molecules without swelling the bilayer, which must mean that the triglyceride chains are mainly parallel to the chains of the polar lipids. In the saltfree system, the triglyceride molecules can enter the gap between the hydrocarbon chain tails of the bilayer and therefore increase its thickness.

The amount of hexagonal liquid-crystalline phase was very small at all water compositions. By ultracentrifugation, this phase separated as a small precipitate at the bottom of the tube, together with contaminating colloidal aggregates of protein and starch granules. We believe that this phase is related to the presence of divalent ions; the presence of traces of divalent ions in an aqueous system of negatively charged lipids favors the formation of the reversed hexagonal phase (Rand and Sen Gupta 1972). A few lipid-

water mixtures with 0.26M sodium chloride in the water also were examined, and it was evident that the relative amounts of the L2-phase and the liquid-crystalline phase changed to some extent, indicating that the small number of divalent ions have a significant effect on the phase equilibria.

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PHYSICAL STRUCTURE AND PHASE PROPERTIES OF AQUEOUS SYSTEMS OF
LIPIDS FROM RYE AND TRITICALE IN RELATION TO WHEAT LIPIDS

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ABSTRACT

In connection with studies of technical functionality of lipids in cereals, the aqueous systems of lipids from rye and triticale flour are described and compared to different wheat cultivars. Rye lipids give a lamellar liquid-crystalline phase with very small ordered regions, and this phase is continuously changed into an L2-phase with increasing water content. Triticale lipids exhibit phase equilibria in between lipids of wheat and of rye. Evidence is given for a significant role of the lamellar liquid-crystalline phase with regard to technical functionality when the cereal flour is worked in water to give a dough.

INTRODUCTION

In previous papers the phase behavior of aqueous systems of lipids from wheat flour and wheat gluten has been reported (1,2). One conclusion from these results was that the equilibria between the different occurring physical structures are very sensitive for small variations in the chemical composition of the lipid mixtures. The functional significance of the different possible lipid-water phases is obvious; lipid-water phases are formed when

a flour is mixed with water, and successive removal of the polar lipids results in loss of gluten functionality, whereas addition of these or similar lipids can normalize a 'delipidized' flour. There are profound differences between doughs made from wheat compared to those made from rye or triticale. The phase equilibria of aqueous systems of lipids from rye and triticale were therefore studied and are reported below. As earlier phase studies concerned a wheat with good baking performance (Amy) a wheat cultivar with poor baking performance (M. Huntsman) was also examined.

MATERIALS AND METHODS

The wheat flour was milled from a winter wheat (M. Huntsman, 1975) to 69% extraction. Triticale flour (Beagle, 1977) milled to 62% extraction was used. The rye cultivar used was Othello, and it was milled to the desired degree of extraction with a disc mill (47%) and a hammer mill (100%). Details on the lipid extraction procedure, determination of the ratio polar to non-polar lipids, thin layer chromatography (tlc), preparation of lipid-water phases and structure analysis of the different phases obtained, have been described previously (1,2).

RESULTS AND DISCUSSION

The phase properties of the system rye lipids-water illustrated as a ternary phase diagram, are shown in Fig. 1. The most important difference between this rye lipid system and those of wheat lipids (1,2) is the relations between the liquid-crystalline phase and the L₂-phase. In the case of wheat lipids (1) there are well-defined limits between the lamellar liquid-crystalline phase and the L₂-phase, and where both phases coexist, they

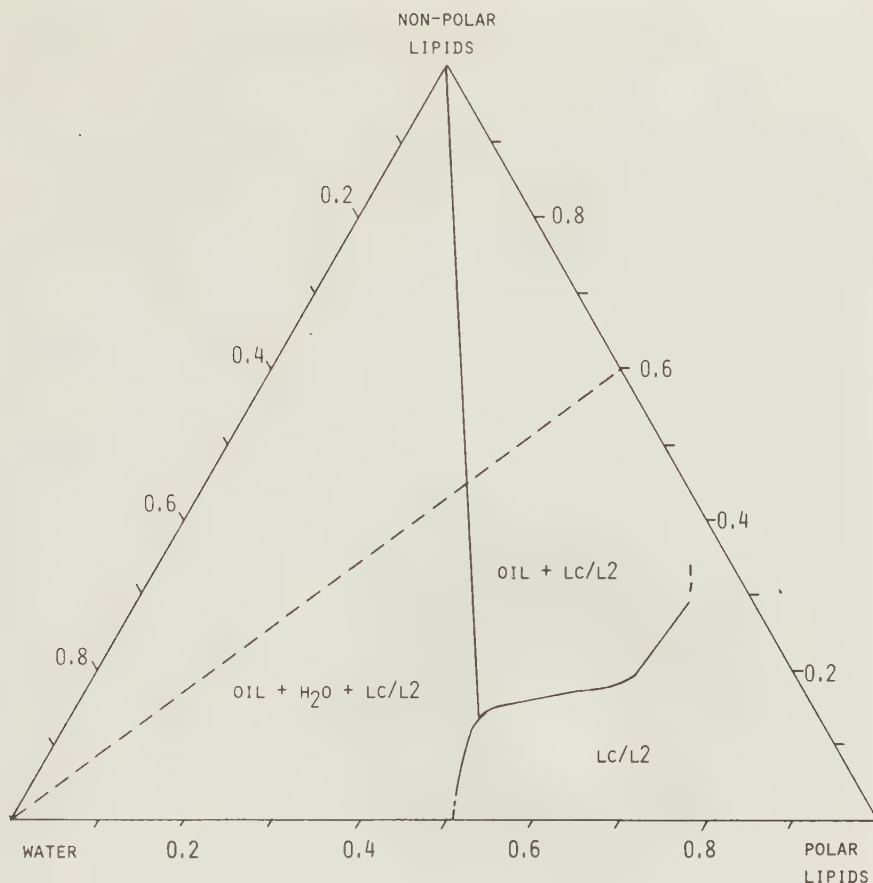


FIGURE 1 Ternary phase diagram of rye lipids (polar/non-polar) and water. LC/L2 stands for the region where the lamellar liquid-crystalline phase changes towards an L2-phase. The broken line corresponds to the polar/non-polar ratio of the rye flour lipids.

can easily be separated. Rye lipids, on the contrary, show a continuous change from a structure of lamellar liquid-crystalline type to the L2-type. At low water content, up to about 20% (w/w) of water, the optical birefringence and the X-ray diffraction pattern correspond to a lamellar liquid crystal. The pronounced line broadening (see Fig. 2) shows that the crystalline regions are very small. When the amount of water is increased there is a

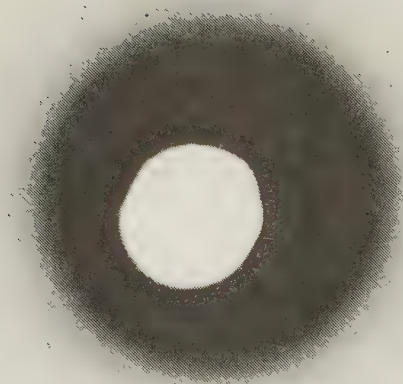


FIGURE 2 X-ray low angle diffraction of the lamellar liquid-crystalline phase (20% (w/w) of water) of rye lipids. The broad diffraction line indicates small domains of the ordered liquid-crystalline phase.

successive loss of optical birefringence and the first order diffraction line is broadened, corresponding to an L2-phase at the highest water content. With regard to the proposed bilayer structure of the L2-phase (1) and the suggested mechanism of transition, involving loss of long-range order (3), it is not surprising that such a transition can take place continuously. A polarizing microscope photograph of the optical texture, where the structure is proposed to be intermediate in relation to the lamellar liquid crystalline phase and the L2-phase, is shown in Fig. 3. There is an interesting two-fold symmetry of the birefringent domains, corresponding to so-called batônnets; characteristic for a lamellar structure.

Rye lipids with extraction grade 47% were also examined and compared with lipids of extraction grade 100% described above. The phase boundaries were somewhat shifted, so that the L2-phase existed to a somewhat higher water content, but the phases behaved in the same way, from a structural point of view, compared to rye lipids of extraction grade 100%. The drastic difference in relation to the wheat lipids (1,2) is thus not a consequence of the higher extraction grade and therefore the higher content of non-polar lipids. Column separation (1) gave a polar/non-polar lipid ratio of 40:60 (w/w) for rye of extraction grade 100% compared to the ratio 48:52 (w/w) of rye of extraction grade 47%. This is quite different from reported values of about 7:93 (w/w) in polar/non-polar lipid ratio (4), but our values are in general agreement with (5).

The relative amounts of polar/non-polar lipids of the extracted triticales lipids are 49:51 (w/w). The lipid pattern is in general agreement with the reported values by Chung and Tsen (5).

The phase diagram of the system triticales lipids-water is shown in Fig. 4, and photographs of the ultracentrifugated samples illustrating the phase equilibria are shown in Fig. 5. A similar successive phase change from a lamellar liquid-crystalline phase to an L2-phase as described in rye lipids is also seen in



FIGURE 3 Optical texture of the liquid-crystalline rye lipids-water phase, with X-ray data shown in Fig. 2.

this system, but not as pronounced. Ultracentrifugation results thus in some degree of separation of L2 and liquid-crystalline

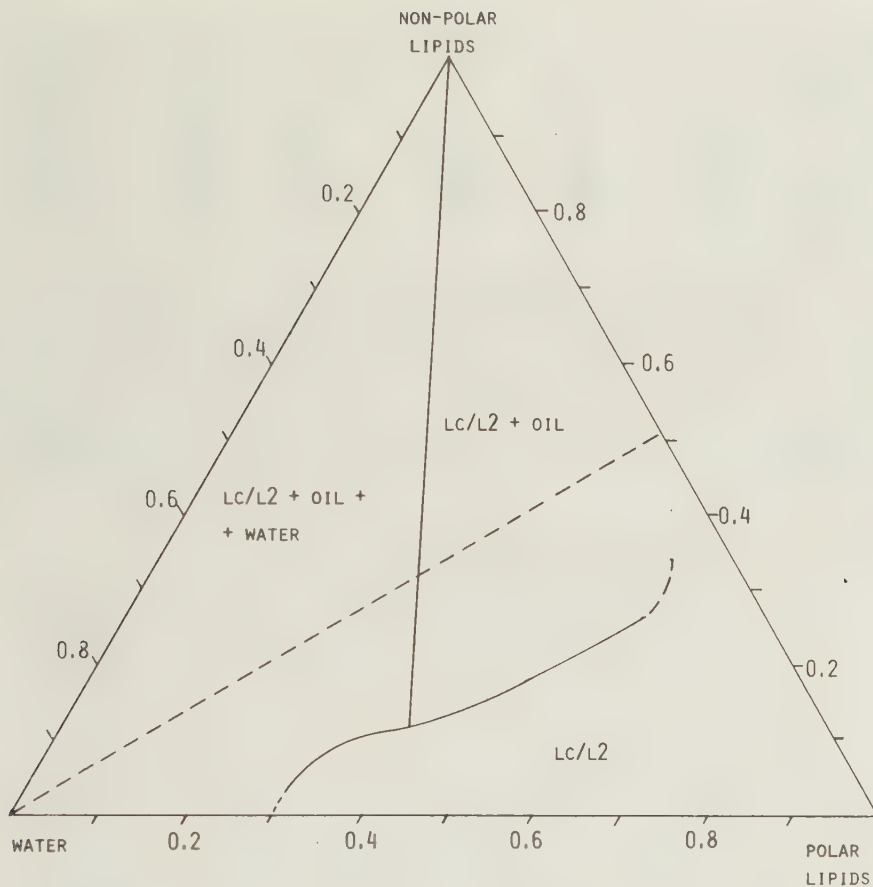


FIGURE 4 Ternary phase diagram of triticale lipids and water. The same nomenclature as in Fig. 1.

phase. The phase properties of triticale lipids are thus intermediate in relation to wheat lipids (1) and rye lipids.

It seemed natural when we started our wheat lipid-water studies to present phase equilibria in ternary phase diagrams with polar lipids (defined as constituents giving aqueous phases) as one component and non-polar (constituents which do not form aqueous phases) as the other lipid component. A complication, however, is that the only experimental possibility to determine the ratio polar/non-polar lipids is based on separation in organic solvents, and this separation can only give approximate

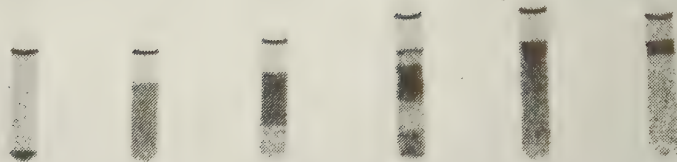


FIGURE 5 Phase equilibria between triticale lipids and water. From left to right 0, 10, 20, 30, 40, 50% (w/w) of water. At 50% (w/w) there is a top phase of non-polar oil, free water in the middle, and a bottom phase of L2 and a small amount of liquid-crystalline phase.

values of polar/non-polar defined strictly according to properties in relation to water. We have examined gluten and flour lipids of several wheat cultivars, and in all cases the amount of non-polar lipids, which separate when water is added, is about the same. This indicates that the corresponding lipid-water system might be meaningful in order to compare phase equilibria, and an advantage is that the uncertainty in polar/non-polar ratio is eliminated. As a complement to such binary systems a simple illustration of the phase equilibria of excess of water is informative. We have chosen to give the equilibria at 70% (w/w) of water, which shows the content of water and non-polar lipids in the aqueous lipid phase.

An illustration of the phase properties of rye and triticale lipids by binary systems is shown in Fig. 6 together with the corresponding diagrams of wheat lipids from Amy and M. Huntsman flour. Fig. 7 shows the corresponding excess-water characteristics.

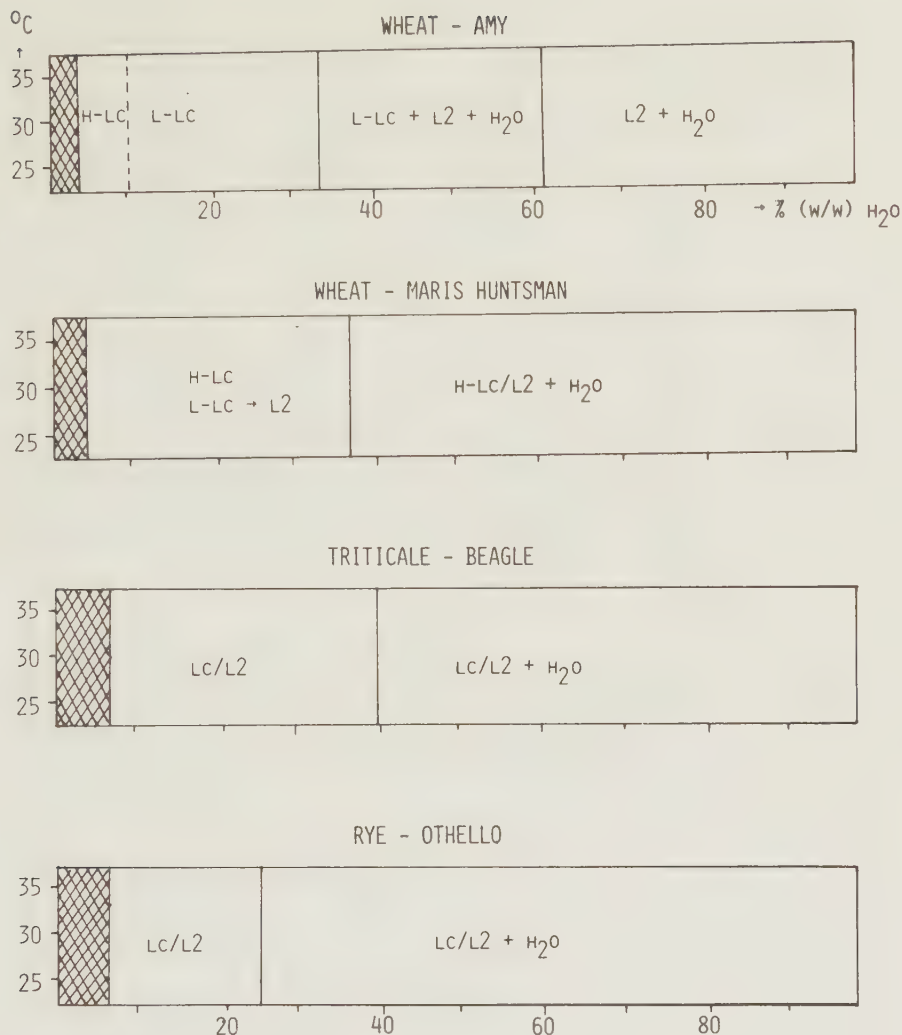


FIGURE 6 Binary lipid-water systems corresponding to lipids extracted from wheat flour (Amy and M. Huntsman), triticale and rye flour. The shaded regions at low water content contains crystalline lipids. Over the whole diagram outside this region, non-polar oil also exists (not indicated in the text). H-LC and L-LC denote hexagonal and lamellar liquid-crystalline phases, and LC/L2 indicates a continuous change from liquid-crystalline phase to L2-phase.

The most pronounced difference between lipids from Amy and M. Huntsman is that the former cultivar forms a lamellar liquid-

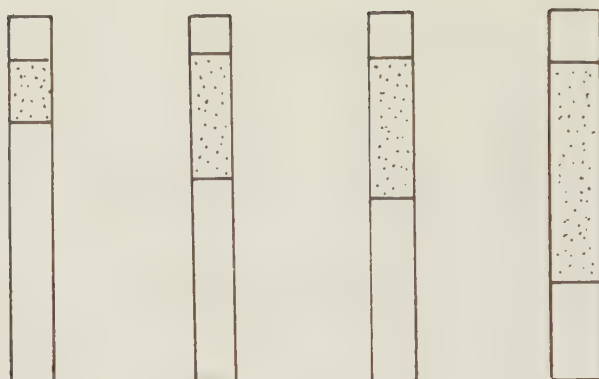


FIGURE 7 Phase equilibria of lipids with 70% (w/w) of water after ultracentrifugation. The top phase is non-polar oil, the middle (dotted region) phase is water and at the bottom a lipid-water phase. From left to right: lipids from Amy and M. Huntsman wheat flour, then lipids from triticale and rye flour.

-crystalline phase over a dominating part of the phase diagram, whereas the hexagonal phase dominates in the case of M. Huntsman. The successive changes in phase equilibria from wheat over triticale to rye are obvious both from the binary systems and from the situation at 70% (w/w) of water.

The gluten lipids from M. Huntsman were also examined and the polar/non-polar lipid ratio was found to be 56:44, which is close to values found in Amy gluten lipids (2). The corresponding ratio of M. Huntsman flour lipids was 63:37, showing a considerably higher amount of polar lipids. We have analysed the starch fraction washed out from this flour and Amy flour, and found that WSB at room temperature (three hours) extracts about twice the amount of polar lipids from M. Huntsman flour compared to Amy flour (3.7 mg lipids/g primary starch in M. Huntsman and 1.0 mg/g in Amy). If the starch fractions are taken out directly from the seeds, however, so that starch damage is avoided, there is no such difference. The higher amount of polar lipids extracted from M. Huntsman flour is thus a consequence of a high degree of damaged starch.

X-ray data recorded from flour and gluten lipids from M. Huntsman were hard to interpret as samples treated as described in (1) were usually non-homogeneous, contrary to the situation with lipids from Amy. The reason is probably the dominating role of the hexagonal liquid-crystalline phase in lipids from M. Huntsman, and phase equilibria involving the hexagonal phase are very slow compared to the lamellar phase. Hexagonal liquid-crystalline phases occur over the whole water composition range in both the flour and the gluten lipid systems. As evident from the hexagonal spacings this phase swells to a high water content. The a-axis is thus 119 Å in the flour lipid system with excess of water, and the corresponding value is 103 Å in the case of gluten lipids. The lamellar liquid-crystalline phase is also observed but to a much smaller proportion.

We have also examined other cultivars, for example Starke, a Swedish spring wheat, which shows a smaller region of existence of the hexagonal phase compared to M. Huntsman, and thus a behavior in between that of Amy and M. Huntsman.

The kinetics of lipid-water interaction might be an important factor with regard to the relative short time involved in dough-baking. The functional lipid structures formed are not necessarily those corresponding to stable phases at the actual water content. When for example 50% (w/w) of water is added to lipids (extracted from M. Huntsman flour) forming a bottom phase in a test tube, the lamellar liquid-crystalline phase is formed at the water/lipid interphase within a few seconds. After a week a film of non-polar lipids is seen on the top, and just below the water layer both L2- and the lamellar liquid-crystalline phases are seen. Still the sample had not reached equilibrium and a zone in the bottom was uninfluenced by water according to observations in the polarizing microscope. It seems probable that an L2-phase, which is proposed to consist of a continuous hydrocarbon chain matrix (cf. ref. (1)), provides an efficient diffusion barrier towards water diffusion. At all studied water proportions (10, 20, 30, 40, 50 and 60% (w/w)) the lamellar liquid-crystalline

phase dominated up to several hours. This indicates that the formation of this phase is favoured when a flour is worked into a dough.

As pointed out earlier (1,2) the physical properties of the lamellar liquid-crystalline phase is directly relevant to functions involved in the formation of gluten properties, whereas the other occurring structures, the L2- and the hexagonal phases, possess no such properties. The lamellar phase can thus expose alternatively a hydrophilic or a hydrophobic surfaces and as a whole phase reduces the interfacial energy of other phases dispersed in the system. Also the mechanical properties of this phase, with very low friction along the planes separating the hydrocarbon chain methyl end groups, favour the formation of dispersion. It is interesting to see that the rye and triticale lipids give no pure lamellar phase, contrary to Amy wheat lipids. The differences observed between different wheat cultivars with regard to the dominance of the lamellar phase are also consistent with a relation between this phase and gluten functionality.

ACKNOWLEDGEMENT

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MONOLAYER CHARACTERISTICS OF A-GLIADIN, A WHEAT STORAGE PROTEIN
AT THE AIR/WATER INTERFACE

by

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SUMMARY

The monolayer properties of A-gliadin were studied at the air/water interface using a continuously recording Wilhelmy type of film balance equipped with a symmetrical compression device. The Π -A isotherms were found to be almost independent on the spreading solutions used (chloroform/methanol/water and water (pH 4)). The isotherms expanded as the substrate were changed from water to a 0.44 M NaCl aqueous solution. The reproducibility of the A-gliadin isotherms was found to be very good, and the standard deviation of the area at a pressure of 12.3 mN/m was $\pm 0.010 \text{ m}^2/\text{mg}$ using a compression velocity of $0.0108 \text{ m}^2/\text{mg}/\text{min}$. A-gliadin produced diluted films if the spreading area used was larger than $0.65 \text{ m}^2/\text{mg}$. This area was termed the minimum spreading area (MSA). The monolayer of A-gliadin was found to be thermodynamical stable during compression up to the collapse point ($25.0 \pm 0.4 \text{ mN/m}$ on 0.44 M NaCl). The equilibrium spreading pressure was equal to the collapse pressure. From the shape of the isotherms two inflections could be identified at $\approx 0.30 \text{ m}^2/\text{mg}$ and $\approx 0.54 \text{ m}^2/\text{mg}$, respectively. The former inflection was identified as the collapse point and the latter as a phase transition of the first order. The first order phase transition was interpreted as the point where the individual molecules condense into a coherent monolayer film. The area position of the Π -A isotherm is discussed in terms of the monolayer thickness, using a minimum protein volume, which was estimated from the amino acid composition. The monolayer thickness at the collapse area, and the phase transition area were found to be $\approx 26.3 \text{ \AA}$ and $14.5 \text{ \AA}$, respectively.

The monolayer behaviour is in principal similar to that of polar lipids with isotherms exhibiting both well defined collapse points and a phase transitions. This is the first time this type of monolayer behaviour has been observed for a protein monolayer. The stable bulk phase in equilibrium with the monolayer after the collapse point is proposed to be a lamellar mesophase.

INTRODUCTION

Wheat storage proteins have a remarkably low solubility in aqueous solutions except at pH-extremes. The solubility of these proteins in aqueous organic solvents like ethanol/water and chloroform/methanol/water, and certain pure organic solvents (i.e. chloroform, methanol) is, however, substantial (Meredith, 1965 a,b; Redman and Ewart, 1973). The surface activity of these proteins is also documented through monolayer studies (Mitchell, 1937; Cockbain and Schulman, 1939). In these earlier monolayer studies, complex mixtures of heterogeneous wheat storage proteins were used, which strongly limit the value of the information which can be obtained. The solubility behaviour of wheat storage proteins resembles that of integral membrane proteins (Gennis and Jones, 1977), and is analogous to that of lipid amphiphiles. Proteins in general contain a high proportion of apolar amino acid residues (between 25 and 50% (Waugh, 1954)), which are more or less evenly distributed along the peptide chain, and these apolar residues are often evenly distributed between the interior and the exterior in the native conformation of the protein (Richards, 1977). Even though proteins in general are classified as amphiphiles, they do not behave as lipid amphiphiles, as this requires strictly asymmetrically localized apolar and polar regions. Such a distribution of the amino acids has been found for integral membrane proteins, e.g. glycophorin (Segrest et al., 1972).

The best characterized wheat storage protein is an aggregating α -gliadin (Bernadin et al., 1967). This protein was later named A-gliadin (Platt et al., 1974). The amino acid composition of A-gliadin is similar to most other wheat storage proteins with about equal amounts of apolar and neutral polar amino acids. The neutral polar amino acids are dominated by glutamine, and the amount of amino acids which can carry charge is extremely low (Bernadin et al., 1967). The primary structure of A-gliadin is not known, the secondary structure in aqueous solution (pH 5) is reported to contain about 30% α -helix and less than 10% β -sheets (Kasarda et al., 1968). The molecular weight of A-gliadin is estimated to 32 000 dalton from SDS-PAGE electrophoresis

(Platt et al., 1974) and amino acid analysis (Kasarda et al., 1968). Any tertiary structure of A-gliadin is not known, however, a compactly folded structure at pH above 5 and at low ionic strengths has been proposed (Kasarda et al., 1976). Furthermore Bernadin (1975) suggests that an aqueous mesophase of A-gliadin can be formed according to its optical properties.

This study was undertaken as a first attempt to characterize the amphipatic behaviour of A-gliadin at the air/water interface. It is likely that the surface behaviour of a protein, which has exposed apolar and polar regions on its surface, should be different from the surface behaviour of normal proteins.

MATERIALS

A-gliadin was isolated as previously reported (Bernadin et al., 1967). All organic solvents used were analytical grade. The sodium chloride used was of suprapure grade (Merck). Double quartz-distilled water was used throughout this work.

The freeze-dried protein powder of A-gliadin had a protein content of 92% as determined by spectroscopy with $E_{1\text{ cm}}^{0.1\%} = 0.58$ at 276 nm (Bernadin et al., 1967), and this method was used for protein concentration in aqueous solution. The protein concentration in the spreading solution used was approximately 0.05% (w/w). Protein concentration in organic solvents was calculated from the weighted amount of protein powder. The following solvents were used; water adjusted to pH 4 with hydrochloride acid and chloroform/methanol/water, 62 : 33 : 5 by weight. The subphase solutions used were double distilled water with and without 0.44 M NaCl adjusted to pH 5 with hydrochloric acid.

SURFACE BALANCE

An automatically recording surface balance based on the vertical Wilhelmy principle was used. The force was measured with a platinum plate (10 x 20 x x 0.1 mm), positioned in the center of the trough, attached to an electronic microbalance (Cahn 2000). The compression device was similar to that described by Andersson et al. (1944) except that two barrier wagons were used in order to produce a symmetrical compression of the monolayer. Asymmetrical compression of a polymer monolayer leads to an asymmetrical pressure gradient in the monolayer with respect to the Wilhelmy plate and such experimental effects were shown by Rosano et al. (1975) for bovine serum albumin. The movement of

the glass barriers (10 x 2 x 200 mm) was connected to a ten-turn potentiometer which provided the potential difference proportional to the movement. The potentiometer was connected to the x-axis of a x-y-recorder. As the signal from the microbalance was fed into the y-axis, the surface pressure area curve was automatically plotted on the x-y-recorder.

The trough (600 x 120 x 15 mm) was made by sandblasting a solid glass plate (620 x 140 x 20 mm). All measurements were performed at room temperature $23 \pm 1^\circ\text{C}$.

EXPERIMENTAL PROCEDURE

The trough, the barriers and the platinum plate were cleaned by a concentrated chromic acid/sulphuric acid mixture, carefully washed with double distilled water and dried before use. The rim of the trough and the barriers were covered with a thin film of paraffin. The substrate was poured in the trough to a height of 1-2 mm above the rim. The surface of the substrate was cleaned by moving paraffin coated barriers from one side of the trough to the other and then sucking out the impurities by a capillary connected to a vacuum pump. The cleaning of the surface was repeated till the surface pressure of the substrate did not exceed 0.2 mN/m as the surface area was reduced by a factor 7. The monolayers were spread evenly on the surface by using an Agla microsyringe. The spreading volume of the solution was about 0.1 ml. The spreading technique used was to very carefully place drops (≈ 0.005 ml) of the solution near the surface and then slowly lower the drop to the interface. This procedure used gave the same result as the technique advised by Trumit (1960). A time interval of 30 min was allowed for the equilibration of the monolayer before it was symmetrically compressed. This procedure gives pressure-area (Π -A) isotherms, whereas pressure-concentration (Π -C) isotherms are obtained by spreading successive aliquots of protein solution on a constant surface area ($8.355 \cdot 10^{-3} \text{ m}^2$). Concentration (C) and area (A) are related via $C = 1/A$. The equilibrium spreading pressure (ESP) is found by adding the protein powder onto a constant surface area till no further increase in the surface pressure is observed.

All Π -A isotherms shown in this study are the original Π -A isotherms obtained experimentally except the isotherm in Fig. 9.

RESULTS AND DISCUSSION

Two aspects of pressure(Π)-area(A)-isotherms, its reproducibility and stability, are of prime importance when monolayer results are to be discussed.

Three surface pressure(Π)-area(A)-isotherms for A-gliadin spread from the chloroform/methanol/water solution on a subphase of water using a compression velocity of $0.0432 \text{ m}^2/\text{mg}/\text{min}$ are shown in Fig. 1. The isotherm shown in Fig. 1b was obtained on the same subphase as the isotherm in Fig. 1a, whereas the isotherms in Fig. 1a and Fig. 1c were obtained from newly prepared subphases. By comparing the isotherms in Fig. 1a and 1c, the reproducibility between two completely independent experiments is illustrated. Since the isotherms in Fig. 1a and 1b were compressed on the same subphase, one would expect any loss of protein to the substrate during spreading and compression to show up in Fig. 1b as a shift in the isotherm towards larger areas. This effect is clearly not observed in Fig. 1, and any error due to spreading losses to the substrate is thus smaller than the overall reproducibility. The reproducibility of the isotherms under the conditions described in this study was always equal or better than the reproducibility illustrated in Fig. 1. The equilibrium spreading pressure (ESP) of A-gliadin on a substrate of water was found to be $20.5 \pm 0.4 \text{ mN/m}$.

Increasing the ionic strength in the substrate to 0.44 gives a significantly more expanded isotherm, which is illustrated in Fig. 2. This effect is probably due to an expansion of the protein molecule at the interface. A decrease of the compression rate by a factor of four gives a slightly more expanded isotherm as can be seen in Fig. 3. The area-scale is expanded in Fig. 3 in order to increase the resolution of the isotherms. It was found that the reproducibility was increased somewhat by using salt in the substrate and by decreasing the compression rate to $0.0108 \text{ m}^2/\text{mg}/\text{min}$. The reproducibility expressed as the standard deviation ($n = 5$) of the area at a pressure of 12.3 mN/m is $0.570 \pm 0.010 \text{ m}^2/\text{mg}$ for isotherms made at the same experimental conditions as in Fig. 3. The A-gliadin isotherm, obtained when $\text{HCl}/\text{H}_2\text{O}$ is used as the protein solvent, is shown in Fig. 4. The reproducibility of the isotherm expressed as standard deviation of the area at a pressure of 12.3 mN/m ($0.591 \pm 0.010 \text{ m}^2/\text{mg}$) is the same for the two solvents used. The area at 12.3 mN/m is found to be expanded $0.02 \text{ m}^2/\text{mg}$ when $\text{HCl}/\text{H}_2\text{O}$ is used as solvent as compared to chloroform/methanol/water, however, the shape of the two isotherms appears identical. Decreasing the compression velocity below $0.0108 \text{ m}^2/\text{mg}/\text{min}$ did not have any significant effect on the isotherms.

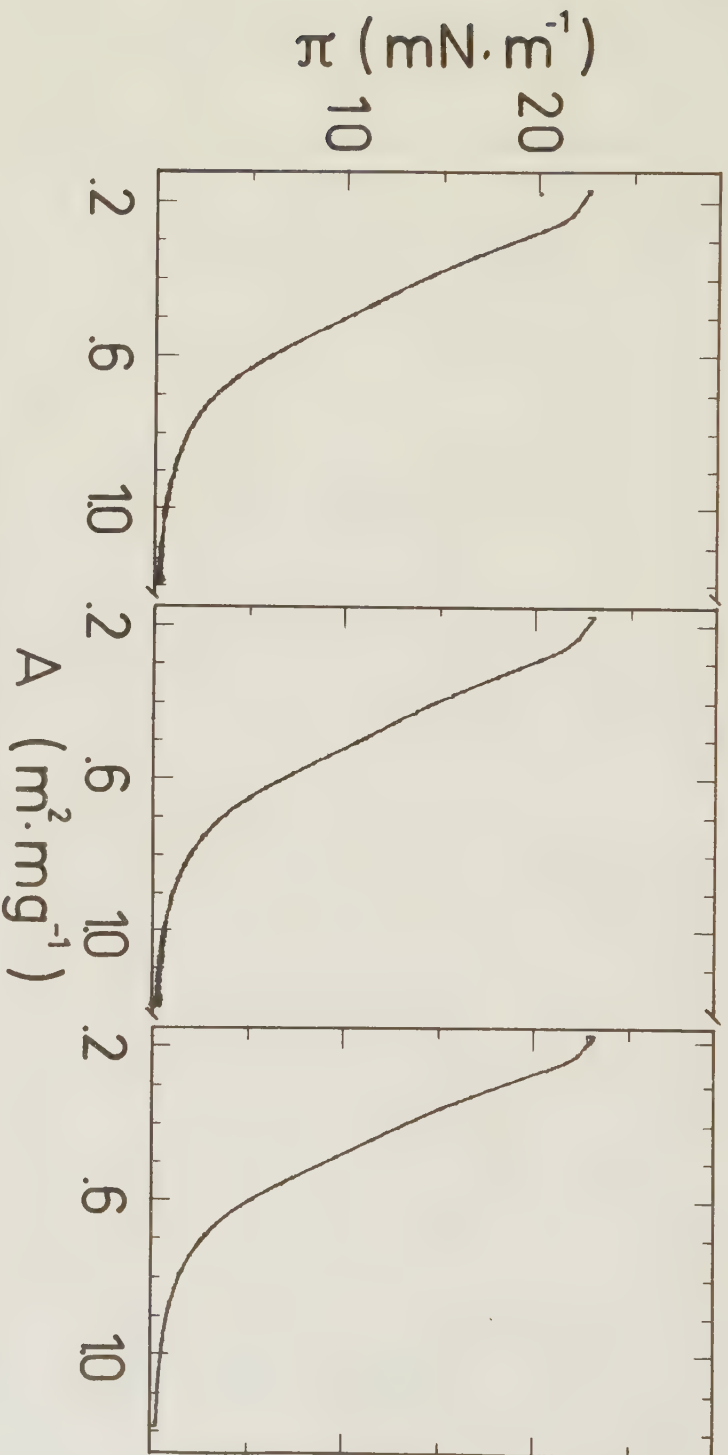


Fig. 1. Π - A isotherms of A-gliadin spread from chloroform/methanol/water on double distilled water is shown. 1a and 1c are identical and the spreading was here performed on new substrates whereas the isotherm in 1b was spread on the same substrate as 1a after cleaning of the surface by sweeping. The compression velocity is $0.0432 \text{ m}^2/\text{mg}/\text{min}$.

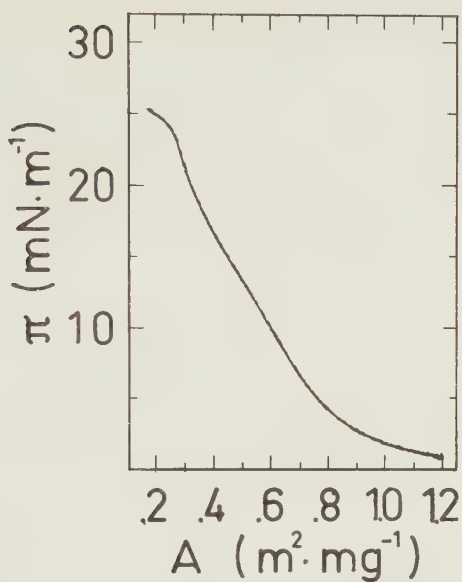


Fig. 2. Π -A isotherm of A-gliadin spread from chloroform/methanol/water on an aqueous substrate with 0.44 M NaCl. The compression velocity is $0.0432 \text{ m}^2/\text{mg}/\text{min}$.

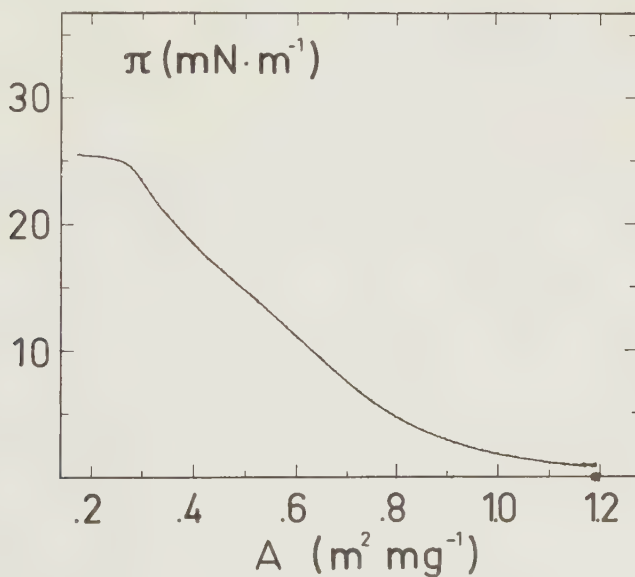


Fig. 3. Π -A isotherm of A-gliadin spread from chloroform/methanol/water on an aqueous substrate with 0.44 M NaCl. The compression velocity is $0.0108 \text{ m}^2/\text{mg}/\text{min}$.

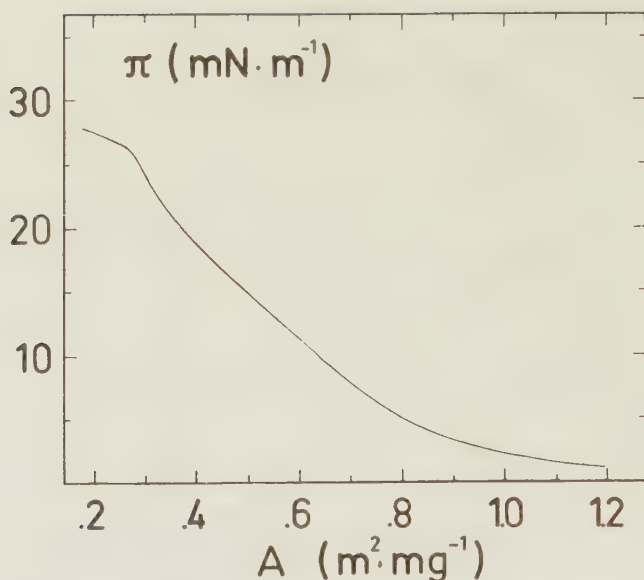


Fig. 4. Π -A isotherm of A-gliadin spread from a water solution (pH 5) on an aqueous substrate containing 0.44 M NaCl. The compression velocity $0.0108 \text{ m}^2/\text{mg}/\text{min}$.

In the results presented above, an initial spreading area of about $1.2 \text{ m}^2/\text{mg}$ is used. It was pointed out by Bull (1947) that, if the initial spreading area is too small, the spreading of the protein molecules is incomplete, resulting in a more condensed monolayer upon compression (i.e. a smaller area at constant surface pressure). Such a monolayer where the molecules are not spread to a maximum area has been called B-films by Joly (1948), whereas films initially spread to a maximum degree were called A-films. The spreading area at which the Π -A isotherms start to depend on the spreading area might be called the minimum spreading area (MSA). This area can be determined by comparing the Π -C-isotherm with the Π -A isotherms provided that the latter is obtained from a spreading area greater than the minimum spreading area. This is illustrated in Fig. 5, where two independent Π -C isotherms are plotted together with a Π -A isotherm. The Π -A isotherm was obtained from a spreading area of about $1.6 \text{ m}^2/\text{mg}$, which is smaller than the spreading area for the previously presented Π -A isotherms. As the Π -A isotherm in Fig. 5 is equal, within the reproducibility of the experiment, to the isotherm obtained at a smaller spreading area (Fig. 4), it is clear that the $\text{MSA} < 1.2 \text{ m}^2/\text{mg}$ for A-gliadin under these conditions. The two types of isotherms in Fig. 5 are equal up to an area of about $0.65 \text{ m}^2/\text{mg}$ and, which thus is equal to the MSA for the A-gliadin. This means that the Π -A isotherms obtained from spreading areas larger than $0.65 \text{ m}^2/\text{mg}$ are equal, and furthermore that these isotherms

are equal to the Π -C isotherm at areas larger than $0.65 \text{ m}^2/\text{mg}$. Under these conditions the A-gliadin monolayer film is of the A-type or diluted type (James and Augenstein, 1966). The character of the concentrated or B-type of monolayer film is poorly defined compared to the diluted film (James and Augenstein, 1966; Yamashita and Bull, 1967). The fact that the conversion of a concentrated film to a diluted film is irreversible (James and Augenstein, 1966) makes it difficult to discuss the concentrated type of monolayer film from a thermodynamic point of view.

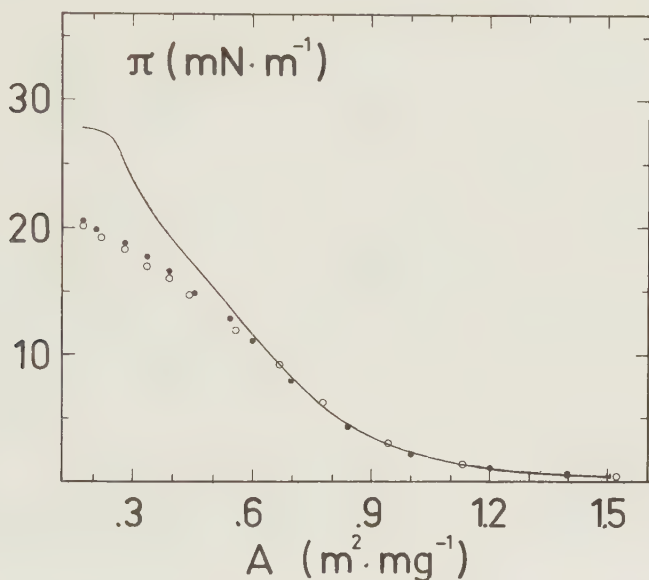


Fig. 5. Π -A and Π -C isotherms of A-gliadin on 0.44 M NaCl. The Π -A isotherm is made as in Fig. 4 except that a larger spreading area was used. The two Π -C isotherms ($\circ$, $\bullet$) were obtained independently.

The thermodynamical stability of a monolayer has been discussed by Gaines (1966). He describes the stability in terms of the equilibrium spreading pressure (ESP), the collapse point (collapse pressure point), losses of the film forming substance by solution and by the existence of internal equilibrium of the monolayer. All these stability criteria except for the ESP can be observed as kinetic effects during the compression of the film. The collapse point is furthermore always observed as an inflection point in the Π -A isotherm. According to the two-dimensional phase rule (Crisp, 1949), the surface pressure should remain constant after collapse (collapse is equal to the onset of bulk phase formation at the surface). The collapse point is often only observed as an inflection in the isotherm due to relaxation

phenomena in the monolayer film from where on the isotherm is flattened out. By making intermittent stops during the compression, and observe the pressure relaxation at constant area, the stability of a monolayer can be observed. Equilibrium or metastable equilibrium prevails in a monolayer if it is compressed by a velocity such that no kinetic effects in the surface pressure are observed at intermittent stops at constant areas. The length of the intermittent stops is critical when this technique is used for stability studies, however. A stop time of 30 min was found suitable for A-gliadin. A π -A isotherm with 13 intermittent stops (indicated by arrows) of an A-gliadin monolayer is shown in Fig. 6. This monolayer was spread from a $\text{H}_2\text{O}/\text{HCl}$ solution on a subphase of 0.44 M NaCl and compressed at a rate of $0.0103 \text{ m}^2/\text{mg}/\text{min}$. No time effects can be observed in this π -A isotherm except at areas smaller than $0.327 \text{ m}^2/\text{mg}$, and the observed onset of surface pressure relaxation is close to an inflection point in the isotherm at $0.295 \text{ m}^2/\text{mg}$. Under these experimental conditions, the A-gliadin monolayer can be said to be stable with regard to the criteria which were given above. The only instability observed is related to the collapse of the monolayer. The ESP of A-gliadin on 0.44 M NaCl was found to be $25.0 \pm 0.4 \text{ mN/m}$ and this pressure is equal to the pressure of the collapse point. This means that the A-gliadin monolayer is in thermodynamical equilibrium with the corresponding stable bulk phase of A-gliadin at the collapse point. The stable aqueous bulk phase of A-gliadin is a mesomorphic phase with a birefringent texture, flow properties and a X-ray diffraction pattern, which resemble those of a lamellar mesophase (Carlson, unpublished results).

Another aspect of monolayer film stability is the degree of hysteresis in the π -A isotherm when the film is subjected to compression-expansion cycles. Such a compression-expansion experiment is shown in Fig. 7. The compression is not continued past the collapse point, since this would have caused a loss of monolayer material to the bulk phase of A-gliadin. In the first cycle the two isotherms are identical, except for a small expansion of the expansion isotherm, which starts at an area between 0.60 - $0.65 \text{ m}^2/\text{mg}$. This expansion in area is about 17% at a pressure of $\approx 2 \text{ mN/m}$. The same hysteresis in the isotherm is also observed if the first compression is stopped at an area of $0.6 \text{ m}^2/\text{mg}$ and then expanded. The isotherm observed during the second compression is identical with the first compression of these isotherms except that the pressure is between 1 and 1.3 mN/m higher over the

whole area range covered. The compression-expansion hysteresis behaviour described is also observed when isotherms are obtained from HCl/H₂O-solution.

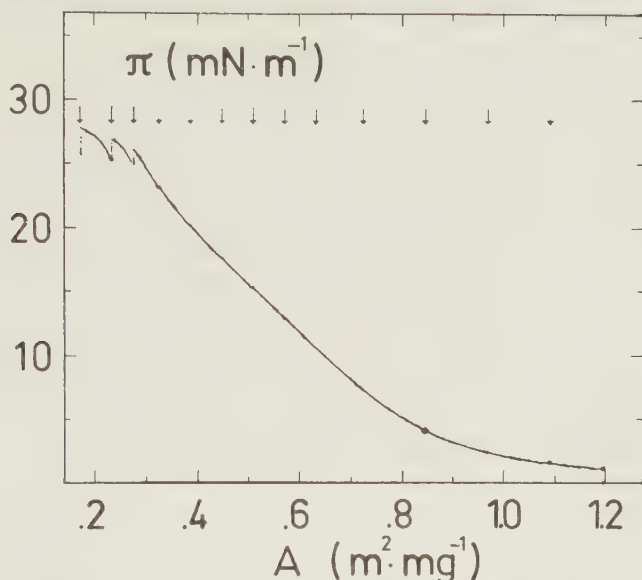


Fig. 6. π - A isotherm of A-gliadin obtained under the same experimental conditions as in Fig. 4 except that 13 intermittent stops ($\downarrow$) of 30 min each were made during the compression.

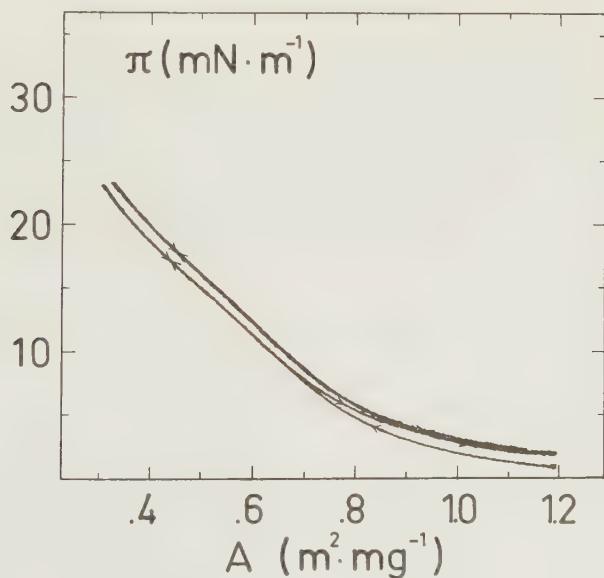


Fig. 7. Compression-expansion isotherm of A-gliadin. The experimental conditions are the same as in Fig. 3. Three compressions and two expansions are shown, and the directions are indicated on the isotherm by arrows.

Physical state

The general monolayer behaviour of A-gliadin described above is rather uncomplicated with respect to both reproducibility and stability, and it resembles the behaviour of simple polar lipids. According to the discussion above it can be stated that the A-gliadin monolayer (Fig. 6) can be regarded as being in a true equilibrium or at least in a metastable equilibrium. With this in mind the shape and position of the Π -A isotherm will be discussed. The shape or form of a Π -A isotherm gives information of the physical states of the monolayer. The general theory of the molecular mechanism of phase transformations in monolayers has been discussed by Dervichian (1939) and Joly (1950). They have shown that the thermodynamic classification of transformations introduced by Ehrenfest for ordinary bulk systems is applicable to the transformations in monolayers, provided that a term for the surface energy is included in the Gibbs free energy G:

$$G = E - TS + pV + \Pi A$$

An ordinary change of state, characterized by a plateau in the Π -A isotherm is said to be a first order transformation, since it corresponds to a discontinuity of the area, A, and of the first partial derivative of G

$$(\sigma G / \sigma \Pi)_{T,p} = A$$

Ordinary changes of state correspond to the equilibrium between two immiscible phases. A second order transformation is characterized by a sudden drop in the compressibility, K, without any discontinuity of the area, and it corresponds to a discontinuity of the second partial derivative of G

$$\left(\frac{\sigma^2 G}{\sigma \Pi^2} \right)_{T,p} = \frac{\sigma A}{\sigma \Pi}$$

$$K = - \frac{1}{A} \cdot \frac{\sigma A}{\sigma \Pi}$$

By calculating $-\frac{dA}{d\Pi}$ from the Π -A isotherm phase transitions can be detected. The calculated quantities of $-\frac{dA}{d\Pi}$ are plotted in Fig. 8 against the area. Two relatively abrupt increases in $-\frac{dA}{d\Pi}$ at $\approx 0.30 \text{ m}^2/\text{mg}$ and $\approx 0.54 \text{ m}^2/\text{mg}$ are observed with decreasing area. These two inflections in the Π -A isotherm thus corresponds to phase transitions of the first order, since in the case

of second order transitions - $\frac{dA}{d\pi}$ would have dropped. From the discussion concerning the stability of the monolayer it is clear that the first order phase transition at $\approx 0.30 \text{ m}^2/\text{mg}$ equals the collapse of the monolayer into its corresponding stable bulk phase. The phase transition of the first order type observed at $0.536 \text{ m}^2/\text{mg}$ is not as distinct as the transition identified as collapse. Theoretically, first order transitions have a plateau in the π -A isotherm as mentioned above which will give $(-\frac{\sigma A}{\sigma \pi})_{T,p} \rightarrow \infty$ in the plateau region. In most practical cases this ideal behaviour is not observed, and $(-\frac{\sigma A}{\sigma \pi})_{T,p}$ usually has a finite value.

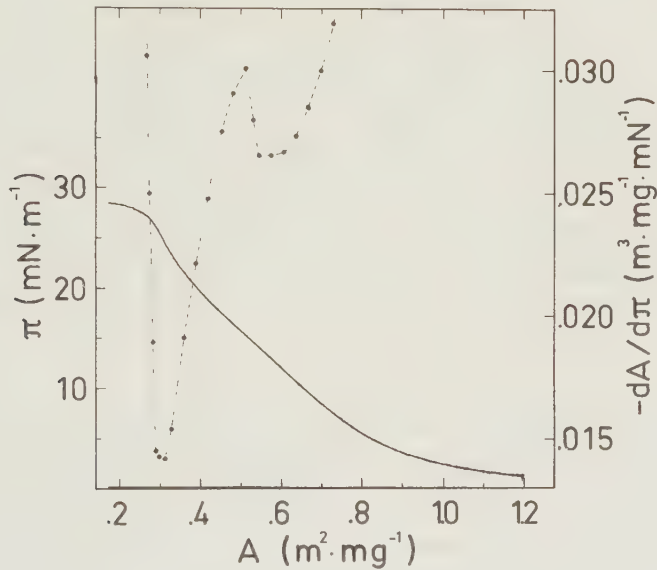


Fig. 8. π -A isotherm of A-gliadin together with the function $-\frac{dA}{d\pi}$. The experimental conditions are the same as in Fig. 4.

The first order phase transition at $0.536 \text{ m}^2/\text{mg}$ resembles a transition often observed in lipid monolayers between a state with liquid chains and a state with solid chains. This phase transition is for example observed for myristic acid (Joly, 1950) and dipalmitoyl lecithin (Phillips and Chapman, 1968) at room temperature. Monolayers of a lecithin at a temperature above its Chapman temperature (T_{Ch}) exists in the so-called expanded state (Phillips and Chapman, 1968). This is also true for natural membrane lipids like digalactosyl diglycerides and phosphatidyl ethanolamines. The observed collapse pressures for all these lipids equal the ESP of the corresponding bulk mesophase at a $T \gtrsim T_{Ch}$. Furthermore the collapse area of the lipid monolayer equals the area

occupied by the lipids in the lamellar mesophases ($\approx 30 \text{ \AA}^2/\text{hydrocarbon chain}$). Aqueous liquid crystalline phases of polar lipids exist only at temperatures where the hydrocarbon chains are in a melted liquid state ($T \gtrsim T_{Ch}$).

Block copolymers which have been observed to form lyotropic mesophases are thought to exist as submicroscopic particles, which constitute the repeating elements (Gallot, 1978). This situation transferred to a monolayer would correspond to a situation where the polymer molecules have lost their identity. From this analogy the phase transition observed in the A-gliadin monolayer isotherm can be regarded as the point where molecules condense into a coherent film, and therefore the identity of the A-gliadin molecules is lost at this point. This coherent monolayer transforms into a three dimensional mesophase at the collapse point. The structure of the mesophase is, as mentioned above, considered to be lamellar. This hypothesis regarding the structure of the A-gliadin mesophase is also confirmed by the fact that the ESP of the A-gliadin coincides with its monolayer collapse pressure. The collapse area and phase transition area for A-gliadin on a pure aqueous subphase are found to be $\approx 0.26 \text{ m}^2/\text{mg}$ and $\approx 0.50 \text{ m}^2/\text{mg}$, respectively, from Fig. 1. The collapse pressure is equal to the ESP also under these experimental conditions.

The relative position of a Π -A isotherm on the area axis is related to the thickness of the monolayer. The thickness of the monolayer can be calculated at a given area, once the volume of the molecules in the film is known. The volume of a protein molecule can be estimated in a number of ways. From densitometric data the apparent specific volume (ϕ_p) can be found. Zamyatnin (1974) has shown that the compositional volume (ϕ_p^*) calculated from the apparent specific volumes of the amino acid residues does not differ very much (within about 3%) from measured ϕ_p values. This comparison included 12 different proteins including both globular and rod shaped proteins. Richards (1974) and Chotia (1975) found that the average packing density of amino acid residues in protein crystals (van der Waal volume/occupied volume) in the interior of proteins is similar to that in amino acid crystals. It is thus clear that the compositional protein volume (ϕ_p^*) can be used as an estimation of the minimum protein volume in bulk.

The compositional protein volume (ϕ_p^*) of the A-gliadin molecule is found to be 0.717 ml/g, using the amino acid composition given by Platt et al. (1974) and the apparent specific volumes of the amino acid residues listed by Zamyatin (1974). Using the molecular weight of 32 000 dalton for A-gliadin (Platt et al., 1974) a minimum molecular volume of 41 882 Å³ is found.

A-gliadin molecules on an aqueous surface will adopt a shape such that the free energy of the system is minimized. This shape probably persists till the molecules become close-packed upon compression. Bull (1947) and Yamashita and Bull (1967) have argued that the area of close-packing is to be found at the area where the compressibility (K) is at minimum. This minimum in K is found at an area of 0.661 m²/mg from Fig. 8, which is almost equal to the minimum spreading area for A-gliadin (Fig. 5), and the interpretation of this area as the area of close-packing according to Bull seems very reasonable. The apparent cross section molecular area at close-packing is then 3.512 Å². If it is assumed that the surface shape of A-gliadin is an oblate ellipsoid, parallel to the interface, the cross-section area of the A-gliadin is found to be 3.160 Å² if the close-packing is hexagonal. The major and minor axes are found to be about 31.5 Å and 10 Å, respectively. Since it is assumed that the protein volume is constant, the shape of the molecule will change upon further compression, resulting in a decrease in the major axis and in an increase in the minor axis. This change of shape of the A-gliadin molecule will continue up to the transition point where the molecule 'loses its identity' as proposed above. At an area (0.54 m²/mg) just before the transition area (0.536 m²/mg) the molecular cross-section area is $\approx$ 2.583 Å², and the calculated major and minor axes are about 28.5 Å and 12 Å, respectively. At an area (0.53 m²/mg) slightly smaller than the transition area, the monolayer is condensed and it is occupying the total surface area. The average cross-section area of the A-gliadin molecule is here $\approx$ 2.816 Å² and the form can be visualized as a hexagon with a side of 33 Å. The average thickness of this monolayer is $\approx$ 14.5 Å. Upon further compression the monolayer thickness will increase till the collapse point is reached at 0.295 m²/mg. The cross-section area at collapse is 1.567 Å²/molecule, which gives a monolayer thickness of 26.3 Å. The calculated geometries of the A-gliadin monolayer are illustrated in Fig. 9.

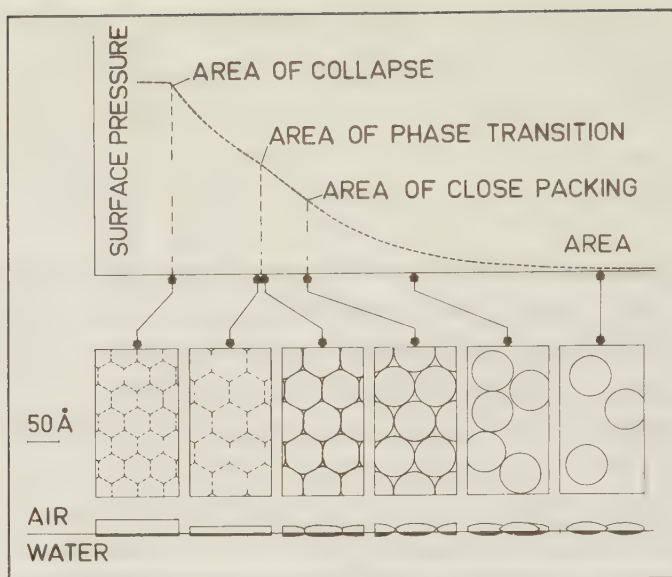


Fig. 9. A schematic picture of the geometry of the A-gliadin monolayer. A π -A isotherm (after Fig. 8) is shown together with the three characteristic areas; Collapse, phase transition and close-packing. The packing of the molecules is illustrated beneath the isotherm both in plane and vertical section.

CONCLUSIONS

The monolayer behaviour of A-gliadin is in principal similar to that of polar lipids, with isotherms exhibiting both well defined collapse points and phase transitions. This phase transition is proposed to mark the point where the close-packed individual A-gliadin molecules are transformed into a coherent homogeneous monolayer. The fact that the collapse pressure equals the equilibrium spreading pressure suggests that the bulk phase structure is related to the molecular arrangement in the monolayer. This bulk structure is proposed to be a lamellar liquid-crystalline phase, which also implies that the A-gliadin molecule has a nonpolar and a polar region on its surface in the bulk phase, and that this asymmetry on the surface of the molecule is not caused by the change in conformation upon spreading.

Acknowledgement

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V

FREE SURFACE ENERGY IN THE ELASTICITY OF WHEAT FLOUR DOUGH

by

T.L-G. Carlson and L. Bohlin

COMMUNICATION TO THE EDITOR

Free Surface Energy in the Elasticity of Wheat Flour Dough

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To understand the importance of changes in free surface energy of dough gas cells in the deformation of wheat flour dough, the following quantities are useful: 1) gas volume of the dough, 2) distribution of dough gas cell radii, 3) distribution of deformation between gas cells and gas-free dough, 4) surface tension between gas cells and gas-free dough, and 5) change in surface area of the gas cells when deformed. Knowing these, one can judge the importance of free surface energy by comparing it in deformation with the energy needed to deform dough elastically.

Baker and Mize (1) and Bloksma (2) demonstrated that up to 20% (v/v) of gas was occluded into wheat flour dough during its mixing. The gas volume in dough used in our study was determined to be 10%. The density value for gas-free dough was 1.24 g/cm³ (1).

We found no data in the literature on the distribution of gas cell radii in dough. The following method was adopted for determining this distribution: slices of dough obtained by microtomy were photographed in a transmission light microscope, enabling us to measure cross sections of gas cells. These cross sections were converted to diameters.¹ Figure 1 shows the observed distribution function of gas cell radii, together with an analytic function fitted to the data. Appendix I gives details of the fitted analytic function and derivation of the ratio of surface area to volume of gas cells. Measuring gas cell cross sections corresponding to a radius of less than ~45 μ m with any certainty was impossible, although the theoretical resolution of cell radius should be ~30 μ m (dough slices 30 μ m thick). From the fitted distribution function, the average surface area per cubic centimeter (A) was calculated to be 41 cm².

Calculation of the change in gas cell surface area resulting from a uniaxial tensile deformation of dough requires an equation relating the strain of the dough to the strain of the gas cells in the dough. Given a homogeneous distribution of identical gas cells of volume fraction v_g , and further that no shear stresses are set up internally during the deformation, the behavior under tensile deformation can be approximated to the schematic drawing shown in Fig. 2.

Since equilibrium requires stresses to balance out internally, two equations follow from Hooke's law:

$$\sigma = E_d \epsilon_d = E_g \epsilon_g \quad (1)$$

where σ is the tensile stress; E and ϵ are Young's modulus and tensile strain, respectively; d is gas-free dough phase; and g is gas phase.

¹Sorenfors, P., Department of Food Engineering, University of Lund, Box 50, S-230 53 Alnarp, Sweden. To be published.

The total strain of the dough is observable. We wish to derive an equation relating the individual strains of the two phases to the observable total strain. This is done as follows, by the definition of strain:

$$\epsilon_{\text{tot}} = \frac{\Delta l_g + \Delta l_d}{l_0} \quad (2)$$

Since the individual strains are given by:

$$\epsilon_g = \frac{\Delta l_g}{v_g l_0}; \quad \epsilon_d = \frac{\Delta l_d}{v_d l_0} \quad (3)$$

it follows by insertion of equation 3 into equation 2 that

$$\epsilon_{\text{tot}} = v_g \epsilon_g + v_d \epsilon_d \quad (4)$$

Unfortunately, information on the relative elastic strength of the gas and gas-free dough is lacking. Sixty percent (v/v) of a dough is made up of starch granules (3,4). This in turn means that in a dough with 10% gas cells, gluten material makes up nearly all the remaining 30%. Starch granules are rigid particles compared to gluten and gas cells. Hence, the gas cells make up 25% (v/v) of the *deformable* material in the dough. Using a value of 1.5 g/cm³ (5) for starch granule density and a specific area of 10,000 cm²/g for starch granules (5), we find a thickness of roughly 0.5 μm of the protein film covering the starch granule and gas cell area. What we term gas-free dough contains starch granules covered with a protein film, and is most likely much more solid-like than the gas phase. If the two phases deform independently as assumed above, the gas cell should show a

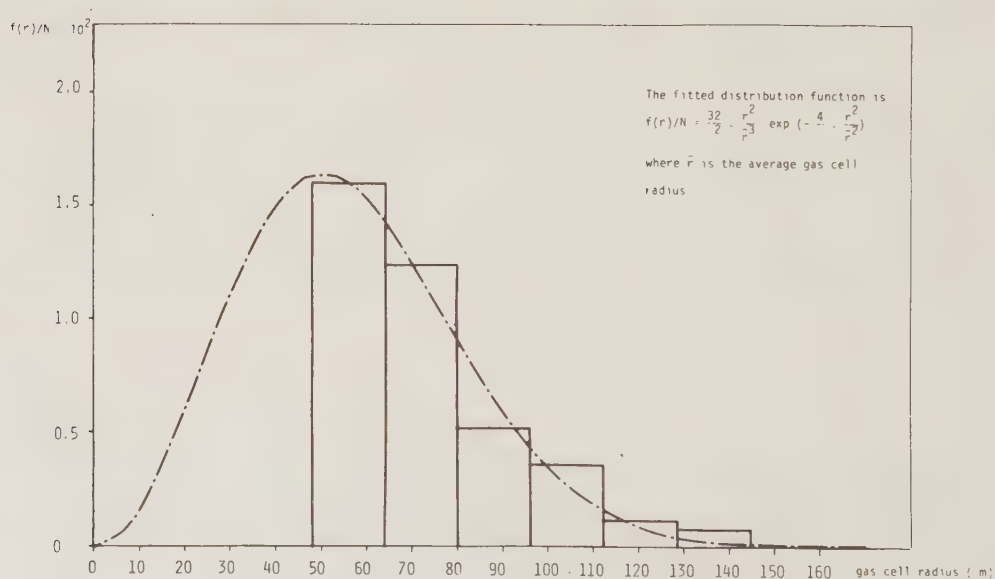


Fig. 1. Distribution of gas cell radii. Columns are measured radius. Curve is fitted distribution function.

much lower resistance to deformation than the gas-free dough. In other words, Young's modulus of the gas cell is likely to be so much smaller than that of gas-free dough that ϵ_d can be neglected compared with ϵ_g (see equation 1). If the volume fraction gas v_g is not too low, equation 4 turns into the useful relationship of:

$$\epsilon_{tot} \approx v_g \epsilon_g \quad (5)$$

A SCHEMATIC PIECE OF DOUGH INCLUDING GAS CELLS

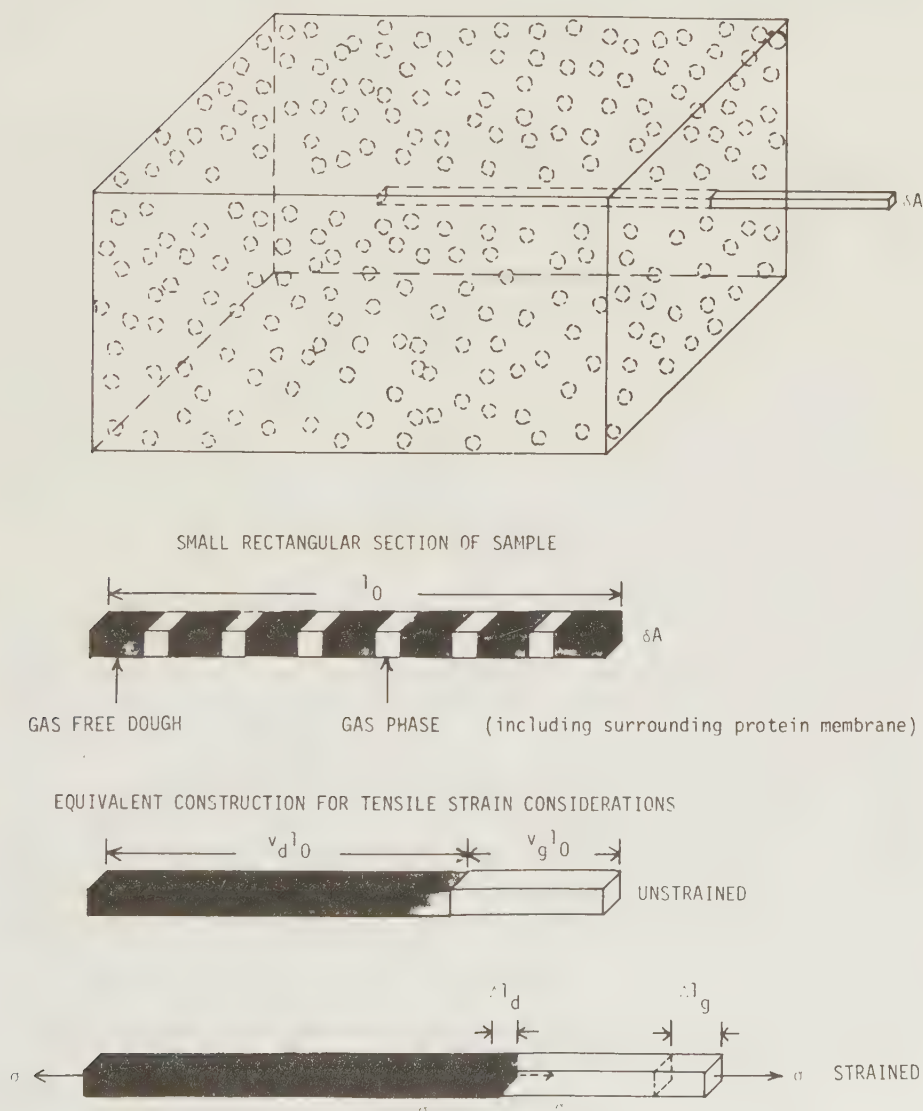


Fig. 2. Schematic picture of uniaxial tensile deformation of dough. v_d and v_g are volume fractions of gas-free dough and gas cells, respectively.

The energy change involved in the deformation of the gas cells is the change in free surface energy, i.e., the product of surface tension and the change in surface area. The surface tension in the membranes surrounding the gas cells is not known. The most correct value to use is the equilibrium surface tension. Tschoegl and Alexander (6) give surface tension area data of gluten, and here we have used the surface pressure value of gluten at the air-water interface at "close-packing," ~ 10 dyn/cm, as an approximate value for the surface pressure of the interface between gas and gas-free dough. This surface pressure corresponds to a surface tension (γ) of ~ 60 dyn/cm at room temperature.

The amount of elastic energy density (energy per unit volume) w , involved in a uniaxial tensile extension of dough is

$$w = 1/2 \sigma \cdot \epsilon \quad (6)$$

where σ is the tensile stress and ϵ is the tensile strain. Data on the tensile deformation of dough (7) reveal that a tensile stress of $6 \cdot 10^3$ dyn/cm² results in a tensile strain of 0.04. The elastic energy density w from equation 6 is 120 erg/cm³.

To calculate the change in surface area of spherical gas cells when subjected to strain, we believe the following is a reasonable assumption: the spherical gas cell deforms into an ellipsoid at constant gas volume where the long axis is equal to $r(1 + \epsilon_g)$ (r is the sphere radius). This model does not overestimate the increase in surface area of the gas cell. The strain of 0.04 of dough with 10% (v/v) gas volume as discussed in the above results in a strain of 0.4 of the gas cells (equation 2). The surface area/gas volume ratio in this case is 4% larger in the ellipsoid compared with the sphere (see Appendix II). This means that the elastic strain in dough gas cells as described corresponds to an energy density of $0.04 \cdot \gamma \cdot A$ equal to 99 erg/cm³. Comparing this with the value 120 erg/cm³ for the total elastic energy density, we conclude that the change in free surface energy accounts for $\sim 80\%$ of the elastic energy involved in deformation of dough.

The temperature dependence of the free surface energy is dependent on how surface tension and surface area vary with temperature. The surface area is insensitive to changes in temperature, whereas the surface tension normally decreases with increasing temperature (8). This means that the deformation increases with increasing temperature at constant stress. This is opposite to the temperature dependence of rubber elasticity. Bloksma and Nieman (9) have found the former temperature dependence for wheat flour dough.

The proposed mechanism is attractive in several respects; it connects rheology with functionality for wheat flour dough, e.g., its gas holding capacity; it makes possible an understanding of how surface active material such as polar lipids improve dough and bread properties of wheat flour. A more rigorous theoretical and experimental study of this subject is currently under way at our laboratory.

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APPENDIX I THE RADIAL DISTRIBUTION

If gas cells with radius r are assumed to form with probability $P(r)$ where

$$P(r) = ae^{-\alpha r^2},$$

the number of gas cells with radius in the interval $r, r + dr$ is given by

$$g(r) dr = 4 \pi r^2 ae^{-\alpha r^2} dr$$

Let N be the total number of gas cells. After normalization, the distribution function has the form

$$g(r) = N \frac{4 \alpha \sqrt{\alpha}}{\sqrt{\pi}} r^2 e^{-\alpha r^2}$$

The average radius is obtained as

$$\bar{r} = 1/N \int_0^{\infty} r g(r) dr = \frac{2}{\sqrt{\pi \alpha}}$$

The average total surface area $\bar{A}$ and volume $\bar{V}$ are given by

$$\bar{A} = \int_0^{\infty} 4 \pi r^2 g(r) dr = 6 \pi N \alpha^{-1}$$

$$\bar{V} = \int_0^{\infty} \frac{4}{3} \pi r^3 g(r) dr = \frac{16}{3} \sqrt{\pi} N \alpha^{-3/2}$$

For a distribution characterized by an average radius $\bar{r}$, the surface area/volume ratio is hence

$$\bar{A}/\bar{V} = \frac{9}{4 \bar{r}}$$

APPENDIX II ELLIPSOID SURFACE AREA

Under uniaxial tensile deformation corresponding to a strain ϵ , a spherical gas cell, radius r , will deform into an ellipsoid with long axis a and the other axis b where,

$$a = r(1 + \epsilon)$$

$$b = r(1 - \epsilon\nu)$$

ν is the Poisson ratio and is taken to be 0.5.

The surface area A_e of the ellipsoid is given by

$$A_e = 2 \pi b \left(b + \frac{a}{\sqrt{1 - b^2/a^2}} \arcsin \sqrt{1 - b^2/a^2} \right)$$

VI

CONE PLATE INSTRUMENT FOR STRESS RELAXATION MEASUREMENTS

by

L. Bohlin, T.L-G. Carlson and G. Bäckström

Cone-Plate Instrument for Stress Relaxation Measurements

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An instrument for the measurement of stress relaxation in shear is described. The force is detected by a sensitive piezoresistive pressure transducer. The requirement of constant strain can be fulfilled within less than 1%. Preset values of shear strain can be imposed on the sample within 0.1 sec. This instrument is suitable for soft solids and elastic liquids with a lower limit of ~ 1 sec for the relaxation time. Results are given for wheat flour dough, a gelatinous deoxycholate complex and a gelatin gel. The instrument is simple, robust, and reliable, as well as inexpensive.

1. INTRODUCTION

In creep experiments a specimen is observed at constant stress but under continuously changing deformation. In stress relaxation experiments, on the other hand, the behavior of a specimen may be studied under fixed deformation. This geometrical constancy permits a more direct analysis of the flow process on the basis of measured data. Soft solids are often subjected to quite large deformations and have generally nonlinear strain (stress) relations. In such cases the strain corresponding to constant stress reflects the nonlinearity in the strain (stress) function as well as the time dependence of strain, and the presence of these two effects simultaneously makes the analysis of the results complex. In a recent theory (1) it is proposed that flow in general is the consequence of cooperative rearrangements in the colloidal structure. The coordination number of cooperative units plays a central role in the theory, and in simple structures it is identical to the structural coordination

number (2). This theory has been developed in great detail for the stress relaxation process, and fitting the theoretical stress (time) relation to measured data one can determine both the coordination number and the strength of the cooperative process.

The condition of a stress relaxation experiment, that the strain be constant during the measurement, is experimentally more difficult to fulfill than the condition of constant stress in the creep experiment. There are in general three ways of keeping constant strain (3): (a) by a servomechanism which continuously adjusts the force as the stress in the sample relaxes, (b) by applying more than enough force, with a stop to limit the deformation, and at intervals balancing the force so that the deformation just clears the stop, and (c) by straining a stiff spring element in series with the sample. Our intention with the present instrument is to study elastic liquids and soft solids that can be moulded in the cone-plate geometry (like gels). Often such systems have short relaxation times (~ 1 sec) and readings taken in

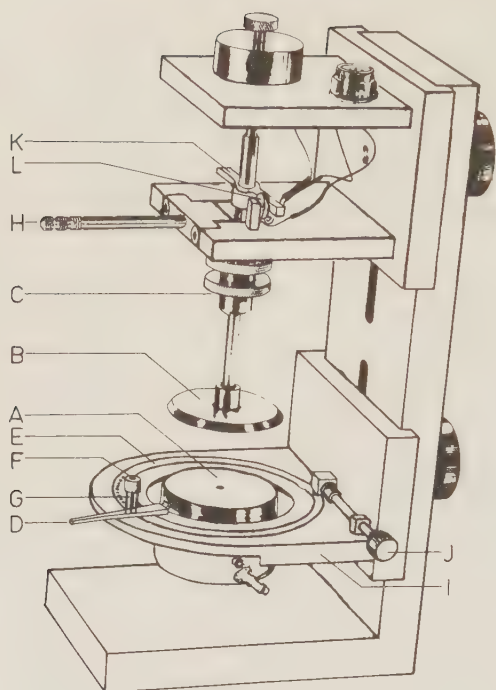


FIG. 1. Schematic drawing of the shear stress relaxation instrument. A, base plate; B, cone; C, locking mechanism; D, lever for rotation plate; E, concentric ring with holes; F, stop plug; G, cylindrical shell; H, arrestment pin; I, fixed table; J, screw; K, torque lever; L, transducer.

tenth of seconds are required and therefore alternatives (a) and (b) above are less attractive. We have chosen the last alternative for the instrument, where the stiff spring element consists of a membrane and a silicon beam in a pressure transducer. The deviation from constant shear strain due to the deflection of the membrane varies with the shear rigidity of the sample but may be kept around 1%. The specimen can be strained rapidly (~ 0.1 sec) to a preset shear strain. The instrument is simple, robust, and reliable, as well as inexpensive.

2. APPARATUS

Figure 1 shows the essential parts of the apparatus with the upper part lifted. (A) is the plane base plate and (b) is the upper

conical piece. The latter is attached to the vertical shaft by means of a locking mechanism (C), which permits convenient change to a different cone angle and/or cone area. The vertical shaft is free to rotate over a small angle due to jewel bearings at the ends. The base plate (A) is circular and may be rotated manually by means of a lever (D). The base plate rests on a concentric ring (E), in which there is a series of holes for a stop plug (F). A cylindrical shell (G), which accurately fits the outside of this plug, is first placed over it and the lever (D) is moved to contact the cylinder. The cone is lifted together with the upper part of the instrument, the vertical shaft being held in place by an arrestment pin (H). The specimen is then placed on the plate (A) and the cone (B) is lowered expelling surplus sample which is removed. The arrestment pin is withdrawn, releasing the cone, and the ring (E) is rotated with respect to the fixed table (I) by means of a screw (J) until there is contact between the torque lever (K) and the transducer (L). The stress relaxation experiment is initiated by the removal of the cylindrical shell (G), determining the shear angle, from the plug (F). The lever (D) is then given a quick turn until it contacts the plug (F). The torque transferred to the shaft by the deformed specimen is recorded as a function of time via the force exerted on the membrane of the transducer. The transducer (L) consists of a membrane, coupled to a pair of piezoresistive semiconductor elements. The two piezoresistors are oppositely influenced by the force and, since they are connected to opposite arms of a Wheatstone bridge, the effect of temperature drift of the semiconductor resistance is largely reduced. The bridge output is calibrated in terms of the torque M by means of weights, string, and pulley. The shear stress is given by

$$\tau = 3M/2\pi R^3$$

where R is the radius of the cone-plate system. The shear strain, γ , may for small

angles be written $\gamma = \alpha/\theta$, where α is the angular displacement of the shaft and θ is the angle between the cone and the plate.

With the present length of torque lever, the bridge output is linear to within 2% up to a value corresponding to 220 g cm (0.0216 N m), which corresponds to ~ 13 mV bridge output. The total starting friction at the bearings is about 0.3 g cm. Since relaxation measurements may extend over several hours, the long time stability becomes important. The average zero drift of the device has been measured and corresponds to 0.3 g cm/hr. This drift may be caused partly by changes in the local atmospheric pressure, which acts on the transducer membrane, partly by temperature change of the transducer. It can be corrected for, however, since a zero reading may be taken after the experiment.

3. RESULTS AND DISCUSSION

To illustrate the applicability and the accuracy of the instrument we now report measurements on three soft materials, a gelatinous complex prepared from sodium deoxycholate (4), wheat flour dough, and a gelatin gel. The shear rigidity of these substances differ by about one order of magnitude and different cone-plate radii must be used in order to operate in the linear range of the transducer where also the shear strain is sufficiently constant. The geometrical data for the cone-plate systems used (5) are given in Table I together with the static stress-strain data. All data on shear strains have been determined by measuring angles of rotation by means of the reflexion of a laser beam by a mirror on the vertical axis.

The deviation from constant shear strain is due to a deflection of the membrane of the pressure transducer. At the torque 50 g cm the deflection leads to a rotation of the vertical shaft through an angle of 0.006° . Specifically, for the two cone-plate systems used in this study a deviation of 1% from

TABLE I

Geometrical Data for the Cone-Plate Systems and Static Stress-Strain Data

	Cone-plate 2	Cone-plate 4
Cone-plate radius (cm)	2.65	1.23
Cone-plate angle (radians)	0.053	0.169
Instrumental constant (dyn/cm ² /mV)	417	4170
Shear strain		
Shell 1	0.201	0.063
Shell 2	0.406	0.127
Shell 3	0.608	0.190
Shear strain		
Cholate gel	—	0.190
Dough	0.201	—
Gelatin gel	0.406	—
Shear modulus (dyn/cm ²)		
Cholate gel	—	105400
Dough	16400	—
Gelatin gel	7200	—
Deviation from constant shear strain (%)		
Cholate gel	—	-0.5
Dough	-2.6	—
Gelatin gel	-1.2	—

constant strain results if the shear modulus of the sample is 6.2×10^3 dyn/cm² for cone-plate 2 and 2×10^5 dyn/cm² for cone-plate 4, respectively.

The cholate gel (45% aqueous solution) was melted in a tube by hot water and poured onto the plate. The cone was lowered into its position and the liquid was allowed to gel at room temperature for about 1 hr. After gelation the measurements were taken. The results are shown in Fig. 2a by filled circles. The derivative of the relaxation curve is shown in Fig. 2b by filled circles.

The dough was prepared from a wheat flour (Starke II) and distilled water in weight ratio 10/5.42 by mixing in a 10-g farinograph for 6 min at 62 rpm. Freshly prepared dough was placed on the plate of the cone-plate system. On lowering the cone the dough fills the gap between the cone and the plate, and expelled dough can be trimmed off with a scalpel. After equilibration for 90 min the measurements were

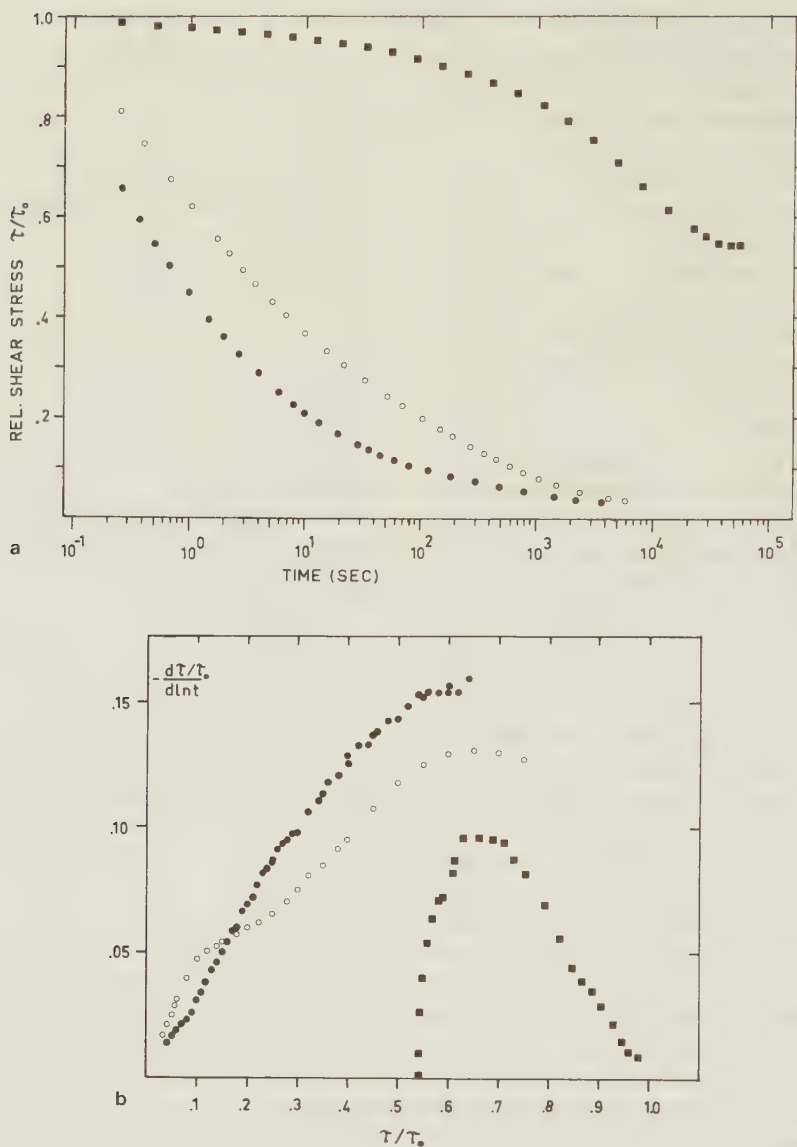


FIG. 2. (a) Normalized shear stress relaxation curve. Cholate gel (●), wheat flour dough (○), and gelatin gel (■). (b) Plot of the derivative of the relaxation data given in Fig. 2a. Cholate gel (●), dough (○), and gelatin gel (■).

taken. The results are shown in Fig. 2 by open circles.

The gelatin gel (5% by weight) was prepared from commercial gelatin powder. The procedure used was the same as for the cholate gel but the gelatine gel was matured for 5 hr. The results of the relaxa-

tion experiment are shown in Fig. 2 by filled squares. The value of the shear modulus of the gel determined after the experiment was twice the value at the start of the experiment.

As is evident from the detail with which the derivative of the relaxation curve can

be obtained, the performance of the instrument for these three materials is quite satisfactory. The range of applicability of the instrument can be extended by changing the length of the torque lever (K).

ACKNOWLEDGMENTS

Helena Ljusberg-Wahren kindly provided the sample of the deoxycholate complex. Lars Hernqvist photographed the instrument.

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VII

SHEAR STRESS RELAXATION OF WHEAT FLOUR DOUGH AND GLUTEN

by

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SHEAR STRESS RELAXATION OF WHEAT FLOUR DOUGH AND GLUTEN

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ABSTRACT

Measurements of shear stress relaxation of wheat flour dough and its corresponding gluten in cone-plate geometry are reported. The range of shear strains used is 0.1–1.0. It is found that the relaxation function is separable into a function of strain and a function of time for shear strains up to 0.4 for dough and 1.0 for gluten. The strain function is non-linear for both dough and gluten. Typically the observed half relaxation time is 1.5 seconds for both dough and gluten. The data suggest the existence of two separate flow processes. The observed time-dependence of the relaxation function has been analysed in terms of a theory of flow as a cooperative phenomenon. In this analysis the process which occurs at short times (0.1–10 s), is characterized by a four-fold coordination of flow units. This process is similar in dough and gluten. The second process, occurring at longer times (10–10⁴ s), is different for dough and gluten and the coordination numbers found are two and one, respectively. This second flow process has to our knowledge not been reported previously.

INTRODUCTION

The rheology of wheat flour dough has been extensively studied. There are, however, only a few fundamental studies. The last review on this subject is by Hibberd and Parker (1975b). They conclude that the results of even the few fundamental studies are often contradictory and do not lead to a consensus as to even the qualitative behaviour of dough. Since this review, Matsumoto (1979) has reported uniaxial tensile stress relaxation measurements on synthetic wheat flour dough and gluten bars supported on a mercury bath. Shear creep and creep recovery of dough in parallel plate geometry have been reported by Hibberd and Parker (1979). Gluten has been studied in uniaxial tensile creep of a bar supported on a mercury bath (Funt Bar-David and Lerchenthal, 1975).

We report in this work shear stress relaxation measurements in cone-plate

geometry on wheat flour dough and its corresponding gluten. The experimental results are discussed in terms of a theory of flow as a cooperative phenomenon (Bohlin, 1980).

THEORETICAL BACKGROUND

Various approaches are available for the theoretical analysis of solid state flow. The viscoelastic properties of polymers are usually described in terms of the theory of relaxation time spectra (Ferry, 1970, p. 60). This theory has also been frequently used in the rheology of viscoelastic foodstuffs (Sherman, 1970, p. 185). In this theory the time-dependence of the stress $\sigma(t)$, is given by

$$\sigma(t) = \epsilon_0 \int_{-\infty}^{+\infty} H(\tau) e^{-t/\tau} d\ln\tau \quad (1)$$

where ϵ_0 is the instantaneous strain and $H(\tau)$ is the distribution of relaxation times. This theory permits a formal description of any relaxation experiment but aids little to our understanding of the nature of the flow phenomenon.

Flow phenomena in metals are often interpreted with the empirical Hooke-Norton law as well as the semiempirical theory of stress-aided thermal activation (Osipov, 1964; deBatist, 1975, p. 71). In the empirical Norton law the rate of stress relaxation is given by the power law

$$\dot{\sigma} = -B(\sigma^*)^n \quad (2)$$

where B and n are constants and σ^* is the "effective" stress $\sigma - \sigma_\infty$. σ_∞ is the stationary stress at long times. One consequence of this law is that, for n greater than one, the 'rate' $d\sigma/d\ln t$ approaches zero linearly in $\sigma - \sigma_\infty$. This is made use of in the method of Li (1967) to extrapolate experimental rate data to obtain σ_∞ . This method has been used both for metals and polymers (see e.g. de Batist and Callens, 1974; Rigdahl, 1976). It appears to us, however, that the functional form of this power law (eqn (2)) is incorrect especially in the vicinity of $\sigma = \sigma_\infty$, where it is actually used. From the theory of fluctuations one would expect a linear dependence, i.e. $n = 1$, in this region. This is also corroborated by carefully analysed experiments (see Bohlin, 1980; Selden, 1979).

The theory of stress-aided thermal activation relates the rate of stress decay to the effective stress σ^* , and an activation volume, v

$$\dot{\sigma} = -A \exp(v\sigma^*/kT) \quad (3)$$

Kubat and coworkers have used a combination of eqns (2) and (3) to describe the stress relaxation of solid polymers (see e.g. Kubat and Selden, 1978). In a recent work (Kubat et al., 1978) they claim that eqn (3) appears to fail to explain experimental findings.

In a recent theory of cooperative flow (Bohlin, 1980) a model is proposed

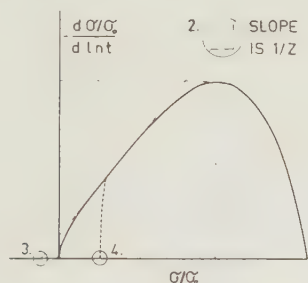
where flow is the consequence of cooperative rearrangements of flow units. In this theory the functional form of the flow equation is determined mainly by the coordination number z of flow units. For stress relaxation, the rate equation describing the relaxation process before the stationary state is reached, is given by

$$\dot{s} = -(1/\tau) \cdot s(s + \alpha)^z \quad (4)$$

where $s = \sigma/\sigma_0$, σ_0 is the initial stress, τ is a relaxation time and α is a measure of the strength of the cooperativity.

The possibility of a direct physical meaning of the coordination number makes this theory attractive. It has been shown (Bohlin and Fontell, 1978; Bohlin, 1979a) that observed oscillatory and steady flow in two simple mesomorphic model systems with two- and sixfold structural coordination, respectively, interpreted with the theory gives a flow unit coordination number z equal to the structural coordination number.

The analysis of the stress relaxation experiment in terms of the theory of cooperative flow is done with the graphical procedure shown in Fig. 1. In the



ANALYSIS OF STRESS RELAXATION DATA

1. CALCULATE $d\sigma/\sigma_0/d\ln t$ VERSUS σ/σ_0 BY GRAPHICAL OR NUMERICAL DIFFERENTIATION OF THE MEASURED σ/σ_0 VERSUS $\ln t$ CURVE

2. DETERMINE THE COOPERATIVE COORDINATION NUMBER z AS THE INVERSE SLOPE OF THE LINEAR PART

3. FIND α FROM THE INTERSECTION $(-Z\alpha/Z+1)$. THE COOPERATIVE ENERGY IS THEN CALCULATED FROM

$$\alpha = 1/(e^{|\epsilon|/kT} - 1)$$

4. A STATIONARY STRESS IS OBSERVED FOR SOLIDS

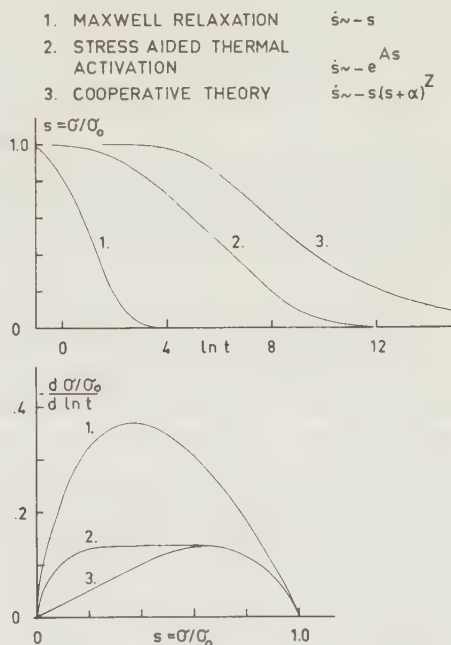


Fig. 1. Theoretical rate—stress plot for a z -fold coordination of cooperative flow units. The method for the analysis of stress relaxation data is given in four steps, indicated by numerals.

Fig. 2. Stress relaxation curves and rate—stress plots are shown for (1) a Maxwell relaxation; (2) the stress-aided thermal activation theory; (3) the cooperative theory. The curve (3) is drawn for $z = 4$ and the maximum rate of curve (2) has been chosen equal to that of curve (3).

case of a strongly cooperative process, $\alpha \approx 0$ and the straight line with the slope $1/z$ intersects the origin. The maximum 'rate' $(d\sigma/\sigma_0/d\ln t)_{\max}$ is $(z+1)^{-1-1/z}$ and occurs at the relative stress $\sigma/\sigma_0 = (z+1)^{-1/z}$. In such case the relaxation curve apart from the stationary state is characterized with the shear modulus, the relaxation time and the flow coordination number.

In Fig. 2 is shown for comparison the theoretical relaxation curves and the corresponding 'rate'-stress curves for a Maxwell relaxation (1), the stress-aided thermal activation theory (2) and the cooperative theory (3). The coordination number in (3) is $z = 4$, and the activation volume in (2) has been chosen to give the same maximum "rate" as of curve (3).

MATERIALS AND METHODS

Dough and gluten were prepared from flour of a Swedish winter wheat, STARKE II (cropped 1975). The wheat was milled to an extraction of 68% (w/w) in a Brabender Quadromat Senior experimental mill. The flour contained 10.0% (w/w) protein ($N \times 6.65$), less than 0.5% (w/w) ash, all calculated on a dry basis. Protein, moisture and ash contents were determined as described in the AACC-methods 46-11, 44-40 and 08-03 (AACC, 1976). The dough composition used was 10.0 g flour on a 14.6% (w/w) moisture basis and 5.42 ml once-distilled water. Flour and water were mixed in a 10-g Farinograph for 6 min at a mixing speed of 62 rpm.

Gluten is the starch free part of the dough and it was obtained by using a semiautomatic gluten washer (Glutomatic, Falling Number, Stockholm, Sweden). Distilled water was used as washing liquid. The recipe used was as in the dough making procedure except that 4.8 ml once-distilled water was used. Both dough and gluten were prepared at $24 \pm 1^\circ\text{C}$.

The stress relaxation measurements were made with a cone-plate instrument which is described in detail elsewhere (Bohlin et al., 1980). The cone-plate radius is 2.65 cm and the cone-plate angle is 0.053 radians. The deviation from constant shear strain due to the deflection of the transducer membrane is 1% per 600 N m^{-2} of shear modulus. All measurements were done at $24 \pm 1^\circ\text{C}$ using freshly prepared dough and gluten. The water content of the gluten samples used was $63.0 \pm 0.2\%$ (w/w). The gluten lump was placed on the tip of a pin and allowed to evaporate surplus of water in the air for 15 min. This was necessary to ensure good adhesion to the metal surfaces of the cone and the plate. The sample was placed on the plate and the cone was lowered expelling surplus material which was trimmed off. The exposed area of the sample at the edge of the cone-plate system was covered in silicone oil to avoid drying. The sample was then allowed to rest for 90 min. During this time the cone axis is free to rotate and some recovery motion may take place. Normal stresses that arose from the shaping of the sample into the cone-plate geometry may relax. These effects were not recorded. The base plate is then rotated so that the lever on the cone axis comes into contact with the pressure transducer. The small stress at contact (Fig. 3; A) will decay due to relaxation and due to

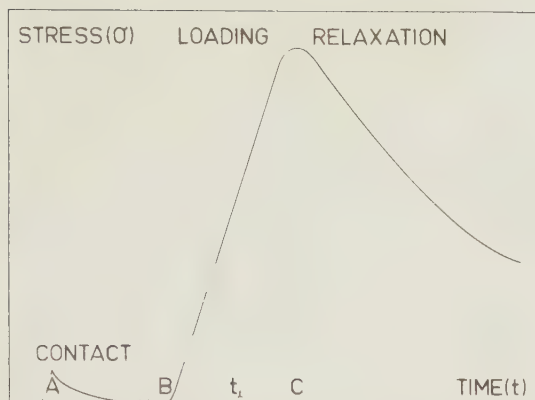


Fig. 3. Schematic drawing of the recorder output in the stress relaxation experiment. A denotes contact of the lever with the pressure transducer.

eventual remaining recovery motion (A—B). σ_A is typically 1% of σ_C and σ_B 1‰ of σ_C . At position B in Fig. 3 the base plate is quickly rotated to give a predetermined strain. The output of the transducer is recorded on a potentiometric recorder (Servogor, Goerz) with a maximum paper velocity of 4 mm s^{-1} and a maximum pen velocity of 50 cm s^{-1} . The typical rise time of the recorder in our experiments is $\sim 0.3 \text{ s}$. This means that the recorded stress will reach its maximum (σ_C) after $t_l \approx 0.3 \text{ s}$ (Fig. 3).

The relaxation modulus $G_{0.3}$ (at 0.3 s) was calculated as $\sigma_C = (\sigma_0)$ divided by the shear strain. The recorded relaxation was plotted on a large sheet of graph-paper ($0.8 \times 1.0 \text{ m}^2$) as the relative stress $\sigma(t)/\sigma_0$ versus the natural logarithm of time. From this graph the flow 'rate' $d\sigma/\sigma_0/d\ln t$ was calculated by numerical differentiation.

The dynamic measurements were performed with a Contraves Balance Rheometer and the procedure was the same as in (Bohlin and Carlson, 1979).

RESULTS AND DISCUSSION

Normalized stress relaxation curves for wheat flour dough are shown in Fig. 4 for shear strains in the range 0.1–1.0. The reproducibility is indicated by the solid vertical bar, which represents the scatter between at least three individual experiments at a fixed strain. This scatter was found to be the same for all shear strains used. It is seen from Fig. 4 that the time-dependence of the relative stress is only weakly dependent on the shear strain for the range of shear strains observed. The observed half relaxation times for dough in the shear strain region 0.1–0.4 range from 1 to 2 s. The relaxation modulus $G_{0.3}$ for dough and gluten is shown in Fig. 5 as a function of strain demonstrating a strongly non-linear behaviour especially for dough. There is a maximum in the stress–strain relationship for dough corresponding to the data given in Fig. 5. The decrease of the stress observed at 0.3 s at increasing strain is due to a decrease of the relaxation time with increasing strain (see Fig. 4).

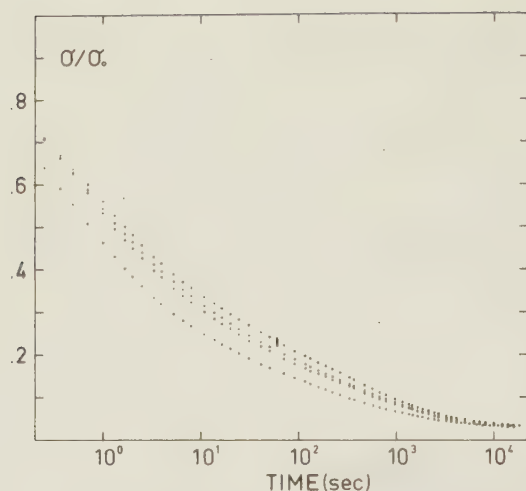


Fig. 4. Stress relaxation curves for wheat flour dough (cultivar STARKE II). The shear strain (γ) and the initial stress (σ_0) for the four curves shown from the top to the bottom are: (i) $\gamma = 0.1$, $\sigma_0 = 310 \text{ N m}^{-2}$; (ii) $\gamma = 0.2$, $\sigma_0 = 378 \text{ N m}^{-2}$; (iii) $\gamma = 0.4$, $\sigma_0 = 464 \text{ N m}^{-2}$; (iv) $\gamma = 1.0$, $\sigma_0 = 410 \text{ N m}^{-2}$.

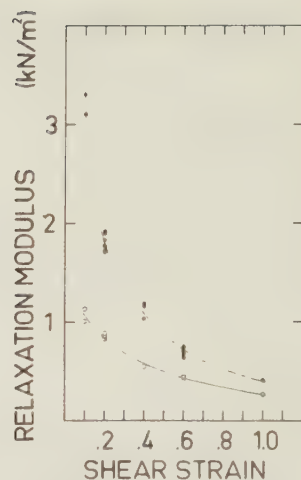


Fig. 5. Shear relaxation modulus at $0.3 \text{ s } G_{0.3}$ are shown for wheat flour dough ($\bullet$) and gluten ($\circ$), as a function of shear strain.

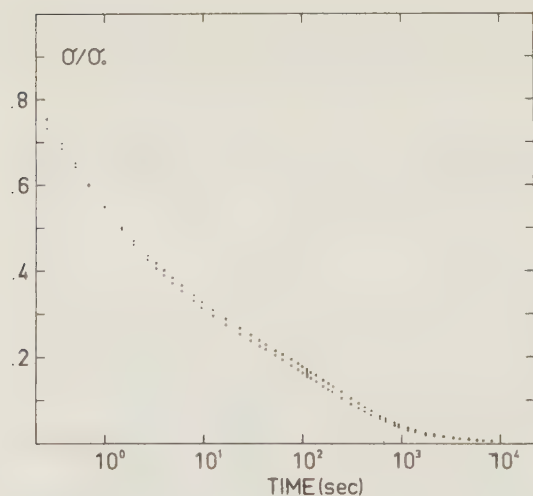


Fig. 6. Stress relaxation curves for gluten at the shear strain 0.2 ($\sigma_0 = 172 \text{ N m}^{-2}$) and 1.0 ($\sigma_0 = 264 \text{ N m}^{-2}$).

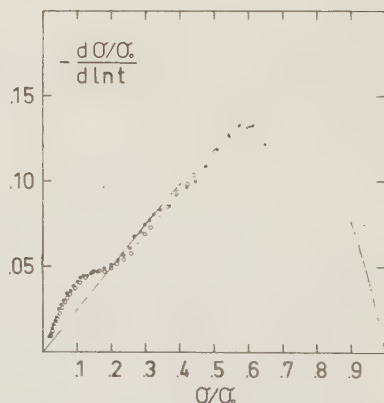


Fig. 7. Rate-stress plot for wheat flour dough obtained from two stress relaxation curves at the deformation 0.2. The solid curve represents the rate-stress plot for a strongly cooperative flow process with a four-fold coordination of flow units.

As the relaxation time decreases an increasing amount of relaxation takes place during the loading period (0.3 s) and an apparently lower stress at 0.3 s is observed.

The relative stress—relaxation curves observed for gluten are independent of the shear strain within the reproducibility in the range 0.1–1.0 and for clarity only the results for the shear strains 0.2 and 1.0 are shown in Fig. 6. The observed half relaxation time for gluten is ~ 1.5 s.

The rate—stress plots ($d\sigma/\sigma_0/d\ln t$ versus σ/σ_0) for dough and gluten are shown in Figs. 7 and 8, respectively. The solid curve represents the theoretical rate stress plot for a four-fold coordination of strongly cooperative flow units ($\alpha \approx 0$). $\alpha < 0.007$ correspond to a value greater than $5 kT$ for the interaction energy between flow units.

The theoretical rate—stress curve agrees with the analysed data for both dough and gluten for relative stress values greater than 0.2, and we shall call this four-fold coordinated flow process the first relaxation process. The average coordination number for both dough and gluten obtained for the shear strains 0.1–0.4 and 0.1–1.0, respectively, is 4.2 ± 0.2 for a strongly cooperative system. For dough at the shear strain 1.0 (see Fig. 4) the observed rate-stress plots are shifted to the right and the linear part intersects the σ/σ_0 -axis at positive values of σ/σ_0 . The theory cannot be applied straightforwardly in this case.

Below $\sigma/\sigma_0 \approx 0.2$ the observed data are anomalous compared to the single process theoretical curve. We interpret this as the existence of a second relaxa-

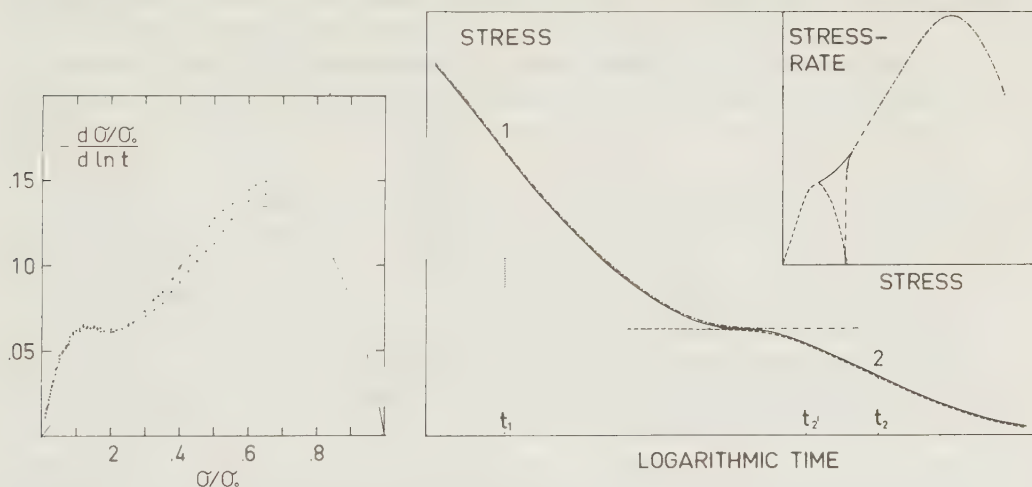


Fig. 8. Rate—stress plots for gluten samples obtained from the stress relaxation curves in Fig. 6. $\gamma = 0.2$ and 1.0 . The solid curve represents the same theoretical flow process as in Fig. 7.

Fig. 9. A hypothetical relaxation curve with two relaxation processes (1 and 2) is illustrated. t_1 and t_2 denote the points of inflexion of the relaxation curve. The corresponding rate—stress plot is inserted.

tion process occurring at long times. A possible relation between the two relaxation processes is proposed in Fig. 9. The two flow processes, denoted by 1 and 2, are shown well separated in time with inflexion points at t_1 and t_2 , respectively. Process number one will relax to a steady stress level which will be the initial stress for the second flow process. The insert shows the corresponding rate—stress plot. If the second flow process would be positioned more to the left, $t_2 \rightarrow t_2'$, the rate—stress plot in the transition region between the two flow processes would follow the solid line. With a relation between the two flow processes described as above it is possible to use the cooperative flow theory separately on the two flow processes. The only parameter we have to add in order to use the theory is the value of the relative stress at which the two flow processes meet. In order to make an accurate rate—stress analysis of this second flow process this part of the relaxation curve has been magnified. Figure 10 shows the second process for dough and gluten at the shear strains 0.2 and 1.0, respectively. The corresponding rate—stress plots are shown in Fig. 11.

The second stress—relaxation process of wheat flour dough shown in Fig. 11 is in good agreement with a strongly cooperative flow process with a two-fold coordination of flow units, starting at a relative stress of 25%. The corresponding flow process for gluten starts at 27% of the initial stress and has a coordination number of one between strongly cooperative flow units. Generally the relative stress at which the second process starts is found to vary between 22% and 27% for both dough and gluten and the average coordination numbers are 2.2 ± 0.2 and 1.2 ± 0.2 for dough and gluten, respectively.

We have measured the storage modulus of gluten at the shear amplitude 0.37 in the frequency range 3–20 rad s^{-1} . The observed frequency dependence was $\sim \omega^{1/3.6}$ and the storage modulus at $\omega = 3 \text{ rad s}^{-1}$ was 400 N m^{-2} .

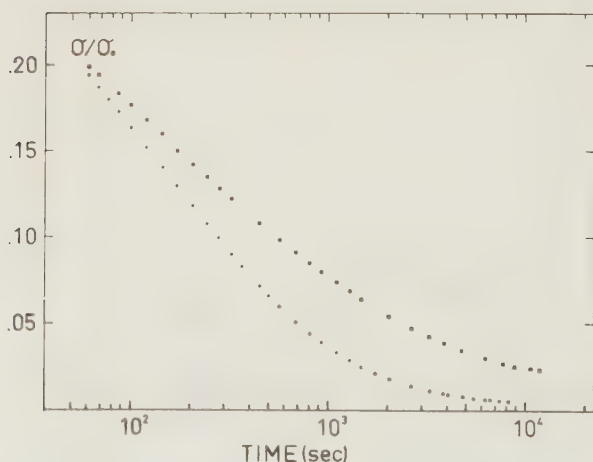


Fig. 10. Stress relaxation data in the low stress region for wheat flour dough (■) and gluten (●). $\gamma = 0.2$ ($\sigma_0 = 378 \text{ N m}^{-2}$) for dough, and $\gamma = 1.0$ ($\sigma_0 = 264 \text{ N m}^{-2}$) for gluten.

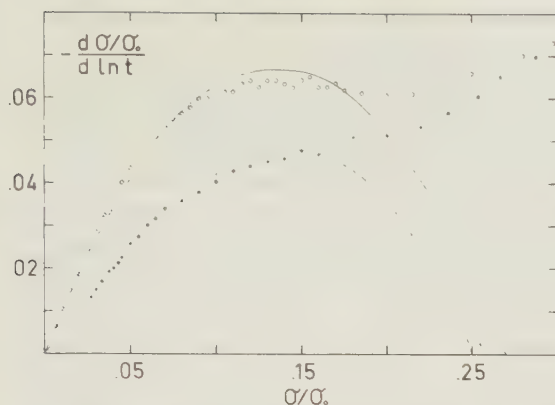


Fig. 11. Rate—stress plots calculated from the data presented in Fig. 10. Dough ($\bullet$), gluten ($\circ$).

Both the wheat flour dough and its corresponding gluten behaved as non-linear viscoelastic materials at the shear strains used in this study in the sense that the relaxation modulus of the two materials were dependent on the magnitude of the strain. The time dependence of the relaxation function was only weakly dependent on the strain for dough and independent of the strain within the reproducibility for gluten. The half relaxation time observed for dough (except at the shear strain 1.0) and gluten was ~ 1.5 s. Our results for the modulus at short times from dynamic tests and stress relaxation are consistent. The storage modulus at the shear strain amplitude 0.37 and at the frequency 3 rad s^{-1} are 400 N m^{-2} for gluten (see above) and 600 N m^{-2} for dough (Bohlin and Carlson, 1979). From Fig. 5 we find by interpolation a relaxation modulus of $\sim 1000 \text{ N m}^{-2}$ and $\sim 500 \text{ N m}^{-2}$ for dough and gluten, respectively, at the shear strain 0.37.

The data for the time dependence of the relaxation function suggest the existence of two relaxation processes for both dough and gluten. One process occurs at short times and the other occurs at long times. According to the cooperative flow theory (Bohlin, 1980), the flow process at short times (0.1–10 s) for both materials can be described as a strongly cooperative flow process with a four-fold coordination between flow units. The flow process at long times (10– 10^4 s), which also is found to be strongly cooperative, shows a coordination of flow units of one for gluten and of two for dough, respectively.

The observation made here concerning wheat flour dough, that the time dependence of the relaxation is approximately independent of the shear strain, is in qualitative agreement with what Hibberd and Parker (1979) found from shear creep of wheat flour dough for stresses up to 60 N m^{-2} and for times less than 250 s. They also found that the dough after a creep time of 10^4 s showed no purely viscous behaviour. This agrees qualitatively with our result that typically 3% of the initial stress remain after 10^4 s.

It is possible to identify the coordination number z of flow units also from other rheological tests than stress relaxation. In a dynamical test the frequency dependence of the modulus is $\sim \omega^{1/z}$ and in a creep test the time dependence of the strain is $\sim t^{1/z}$ (Bohlin, 1980). The dough system used in this study was earlier studied in a dynamical test (Bohlin and Carlson, 1979) and the coordination number was found to be between three and four in the frequency range 1–20 rad s⁻¹. We have analysed the creep data on wheat flour dough given by Hibberd and Parker (1979) using the theoretical expression $t^{1/z}$ (see above) for the time dependence of the strain. Their data at times between 3–20 s give $z = 4$. The four-fold coordination number of flow units in gluten observed in this study both from stress relaxation and dynamical data is also evident from the dynamical data reported by Smith et al. (1970). When analysing their dynamical data we found z to be 3.6 in the frequency range corresponding to the time range where the four-coordinated process was found for gluten in this study. It seems likely, hence, that the four-coordinated flow process in wheat flour dough and gluten is insensitive to the type of flour used and the conditions used for the dough and gluten preparation. The flow process occurring at long times with two- and one-coordinated flow units for dough and gluten, respectively, has not yet been identified in any other reported experiment.

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Note added in proof

The second flow process in gluten (see Fig. 11) may alternatively be analyzed as a less strongly cooperative process with a twofold coordination. The data given in Fig. 11 then correspond to the parameter values $z \simeq 2$, $\alpha \lesssim 0.3$

VIII

DYNAMIC VISCOELASTIC PROPERTIES OF WHEAT
FLOUR DOUGH - DEPENDENCE ON MIXING TIME

by

L. Bohlin and T.L-G. Carlson

Dynamic Viscoelastic Properties of Wheat Flour Dough: Dependence on Mixing Time

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ABSTRACT

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The dynamic rheological properties of wheat flour dough as a function of mixing time are reported for two wheat varieties, Amy and Starke, with strong and medium baking strengths, respectively. The rheological measurements were done with a balance rheometer (Contraves, Zürich) at a shear strain amplitude of 0.37 in the frequency range 0.08–20.9 rad s⁻¹. The

result was evaluated according to a recently developed cooperative flow theory. Differences between a strong (Amy) and a medium (Starke) baking flour were observed. No simple relation was found, however, between the dynamic data's dependence on mixing time and the farinogram.

Flour baking performance has commonly been judged from rheological measurements (Bushuk 1975). The rheological methods are usually empirical, i.e., they do not involve the measurement of a well-defined physical quantity. If such an approach is to be successful, many types of empirical tests must be

performed and correlated with a technical test (e.g., baking). Fundamental rheological tests, on the other hand, are seldom used when judging flour baking performance. No fundamental study has, to our knowledge, been published on dough rheology in which an empirical rheological test or baking test is compared with a fundamental rheological test. The first fundamental rheological measurements of wheat flour doughs in shear were reported by Bloksma (1962). The most extensive works on fundamental rheological properties of wheat flour systems have used dynamic

viscoelastic methods. They have investigated linear behavior (Hibberd and Wallace 1966), influence of water content (Hibberd 1970a) and protein starch content (Hibberd 1970b), and nonlinear behavior (Hibberd and Parker 1975b) of dough systems. Fundamental dough rheology has recently been reviewed by Hibberd and Parker (1975a).

In this work we report the first dynamic measurements of the dependence on mixing time of the rheological properties of wheat flour dough. One would expect that at the relatively high frequencies involved in a dynamic experiment, the rheological properties of the dough would be less significant for judging baking quality than at the very low shear rates involved in the fermentation process (Blokma 1962). We conclude from this work, however, that the dependence of the dynamic modulus on mixing time is distinctly different for a standard bread flour (a winter wheat) and a strong bread flour (a spring wheat). No apparent correlation with the farinograms was found, however. The observed behavior is discussed in relation to the colloidal structure of wheat flour dough.

MATERIALS AND METHODS

Materials

Untreated samples of a spring wheat (Amy, 1975 crop) and a winter wheat (Starke II, 1975 crop) were experimentally milled in a Brabender Quadrumat Senior with extractions of 65 and 68% (w/w), respectively. Chemical analysis of the spring and the winter wheats gave the following contents, calculated on a dry basis (w/w): protein 13.5 and 10.0% ($N \times 5.7$), respectively; ash less than 0.5%. AACC methods (1976) were used for all chemical analyses.

Preparation of Dough

All doughs were mixed at a speed of 62 rpm in a 10-g farinograph mixing bowl at 25°C. The procedure of Voisey et al (1971) was used

with variation of mixing time. The water was added by pipettes accurate to 5 μ l. Doughs were mixed with a water content corresponding to water absorption by the constant flour weight method (Blokma 1962). Water absorptions were 58.6% for Amy and 54.9% for Starke II.

Balance Rheometer Measurements

The balance rheometer (Contraves, Zürich) consists of two concentric spheres rotating with the same angular velocity, ω , about two axes that pass through the center of the spheres. The angle between the rotating axes is small ($<6^\circ$), and the maximum angular velocity is ~ 20 Hz. The sample is contained in the gap between the spheres, and the dynamic modulus is determined by measuring the couple on the inner sphere about two mutually perpendicular axes (Jones and Walter 1969). The rheometer was zeroed without sample at zero angle. About 5 g of freshly made dough was centered on the top sphere. The sample was spread evenly with a spatula, and the top sphere was inserted in its measuring position. The dough was worked for 5 min at an angle of 1° (shear amplitude 0.37) at a frequency of 3.14 rad s^{-1} before the start of the measurement. The deviation from zero torque at zero angle at 3.14 rad s^{-1} was noted. All measurements were done at an angle of 1° and a temperature of $25 \pm 2^\circ \text{C}$. The loss torque was first measured from low to high frequency, and the same procedure was then repeated for the storage torque. After running the highest frequency, a low frequency was measured in order to check any

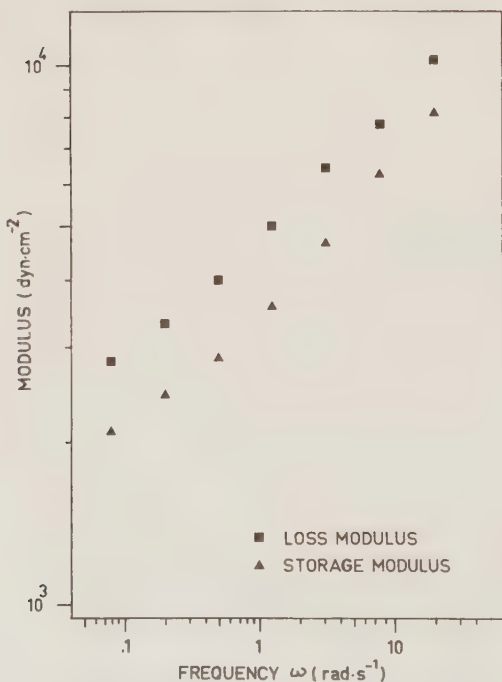


Fig. 1. Frequency dependence of the dynamic modulus for wheat flour dough (Starke), mixed for 8 min at 25°C. The shear amplitude is 0.37.

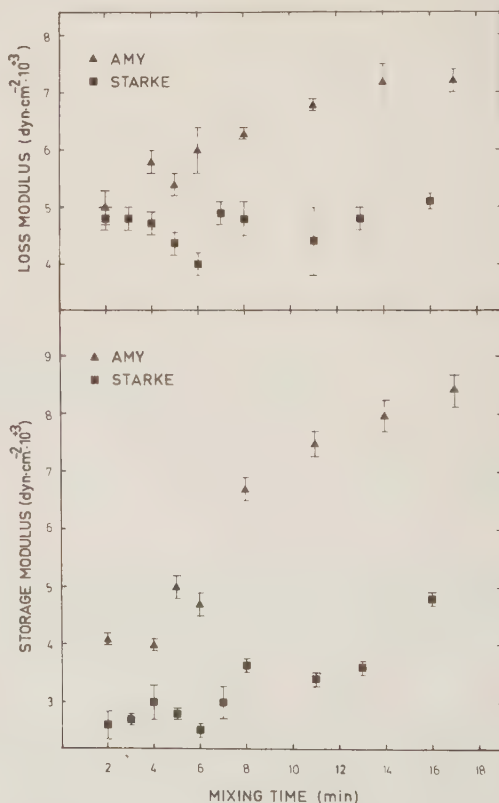


Fig. 2. Loss and storage moduli at $\omega = 1 \text{ rad s}^{-1}$ as a function of the mixing time for flour varieties Amy and Starke. Error bars show standard deviation from mean value of at least three measurements.

hysteresis effect. We have noticed, when doing shear stress relaxation experiments on dough, that putting a dough piece in a measuring cell without imposing stress on the dough is very difficult. Typically a stress will relax to 10% of its initial value in about 1 hr. We have therefore done all measurements at a shear strain amplitude of 0.37, at which the stress from the insertion of the sample is negligible. Because dough is not a rheological linear material at such strains (Hibberd and Parker 1975), the response in stress to a sinusoidal deformation is not strictly sinusoidal but contains higher harmonics. Our analysis of the response in terms of in-phase (storage modulus) and 90° out-of-phase (loss modulus) contributions is approximate, and the results represent averages, including the effect of the nonlinearity.

Reproducibility of Experiments

Great care must be taken in fundamental experiments on biological samples, particularly if small-scale preparations of heterogeneous materials are used. In dough from 10 g of flour, the amounts of water and flour must be known very accurately (Voisey et al 1971) in order to keep the farinograph consistency constant. The procedure is important, as is the insertion of the dough into the measuring cell. Two aspects are critical in this respect: the amount and symmetry of dough in the measuring cell and the dough's stress history. The former problem is a special one of the balance rheometer because surplus dough cannot be controlled accurately. The latter problem is solved by allowing the dough to relax from induced stresses in the measuring cell before the start of the measurements.

RESULTS AND DISCUSSION

The measurements were done in the frequency range 0.08–20.9 rad s⁻¹. Figure 1 shows the frequency dependence of the modulus

for Starke dough mixed for 8 min. The behavior is typical for both flours and for all mixing times. The results at lower frequencies are less accurate because the measured torque may be the same magnitude as the torque at zero angle. We have therefore concentrated our evaluation on the high frequency region, where the double logarithmic plot is approximately linear. Runs in which a hysteresis effect larger than 5% was found were discarded. We have analyzed our data according to a theory of flow (Bohlin 1980) in which the main parameter is the coordination number (*n*) of cooperative flow units. From this theory, the frequency dependence of the dynamic modulus is qualitatively given by the equation:

$$G(\omega) = A \cdot \omega^{1/n}$$

The measured data are represented in this form with:

ω = frequency (rad s⁻¹)

G = modulus (dyn/cm²)

$1/n$ = slope of linear portion of log G vs log ω

A = flow coefficient

In the following text, the storage and loss moduli are designated by G' and G'' , respectively. The coordination number is used to discuss the structure that gives the rheological behavior. Bohlin (1979) has experimentally shown that, for simple well-defined amphiphile-water systems, n is in agreement with the coordination of structural units in the system. For the lamellar liquid crystalline system, n was two and for the hexagonal liquid crystalline system, six.

The relationship between G and the mixing time is given in Fig. 2. Generally the modulus increases with mixing time, with the exception of the loss modulus for Starke. This increase is pronounced for Amy and weak for Starke. The same trends are observed in the n parameter given in Fig. 3. Both n' and n'' increase significantly for Amy whereas no significant mixing dependence is observed for Starke dough. Note that $n' \approx n''$. The magnitude of the modulus at high frequencies is consistent with values reported by Bloksma (1962) from creep tests and with Hibberd and Parker's dynamic data at large amplitudes (1975).

The dynamic data on wheat flour dough (Fig. 3) indicate a structure having approximately a fourfold coordination of flow units in the frequency range studied. As a first step, the flow behavior of dough must be understood on the colloidal level. From a colloidal point of view, dough consists of a continuous aqueous protein-lipid gel with dispersed phases of starch granules and air cells. The continuous aqueous protein-lipid matrix with dispersed gas cells is called gluten. Analysis of stress relaxation experiments

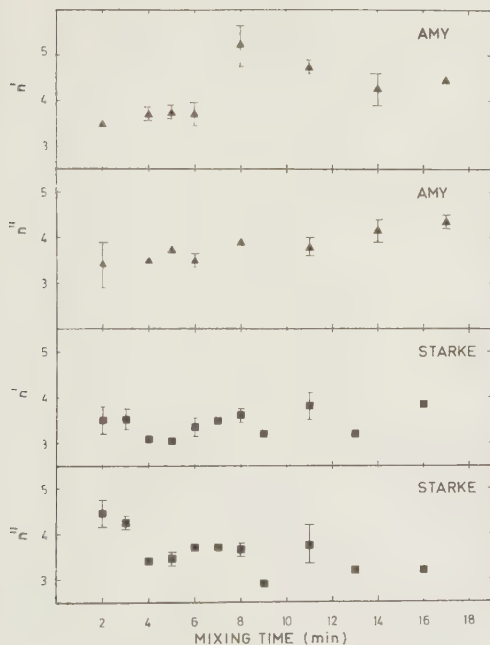


Fig. 3. Dependence on mixing time of the coordination number, n , of a cooperative flow unit for flour varieties Amy and Starke. n' = storage modulus, n'' = loss modulus. Error bars show standard deviation from mean value of at least three measurements.

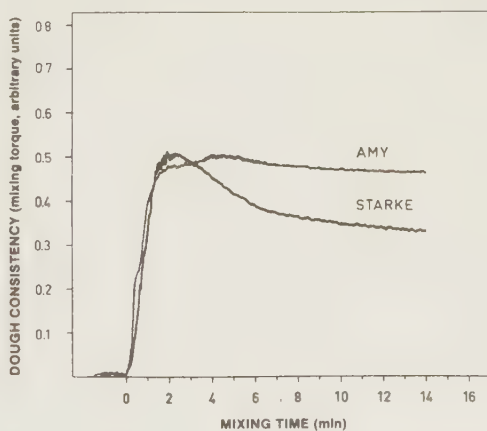


Fig. 4. Farinogram (10-g bowl) for wheat varieties Amy and Starke. Mixing was done with a water content corresponding to the water absorption (Bloksma 1962).

on dough (Bohlin and Carlson 1980) shows two flow mechanisms, one at short times (~ 1 sec) with a fourfold coordination and one at longer times (~ 100 sec) with a twofold coordination.

We have analyzed the recent creep data on wheat flour dough by Hibberd and Parker (1979) according to the flow theory (Bohlin 1980). From those creep data a fourfold coordination flow process can be identified at times corresponding to 3–20 sec. This fourfold cooperative flow process in dough, which occurs at the time scale of seconds, must be regarded as well established; it is found in three different fundamental rheological experiments. The relation between this cooperative coordination number of flow and the colloidal status of the dough has not been investigated. Dynamic data on reconstituted doughs (Hibberd 1970b) imply, however, that the cooperative coordination number increases with increasing starch content. Further experiments are required for an understanding of the relative importance of the dispersed phases in this respect.

We believe the observed differences in modulus as a function of mixing time must be related to the colloidal status of the doughs. In a recent work (Carlson and Bohlin 1978), we proposed that a major part of the elastic energy involved in the immediate deformation of dough appears as surface energy at the gas-gluten interface. This implies that the storage modulus at high frequencies should increase with increasing surface area, assuming constant surface tension. The observed increase of the storage modulus with mixing time for both Amy and Starke may be partly accounted for by an increase in surface area. We have not found any simple relation between the dynamic flow and farinogram consistency (Fig. 4) as a function of mixing time.

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SCIENCE IS A SOPHISTICATED PLAY
WHERE THE TRUTH OF YESTERDAY
BECOMES THE LIE OF TODAY

